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Advancing water absorption capacity in hard winter wheat using a multivariate genomic prediction approach

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Abstract
The water absorption capacity (WAC) of hard wheat (*Triticum aestivum* L.) flour affects end-use quality characteristics, including loaf volume, bread yield, and shelf life. However, improving WAC through phenotypic selection is challenging. Phenotyping for WAC is time consuming and, as such, is often limited to evaluation in the latter stages of the breeding process, resulting in the retention of suboptimal lines longer than desired. This study investigates the potential of univariate and multivariate genomic predictions as an alternative to phenotypic selection for improving WAC. A total of 497 hard winter wheat genotypes were evaluated in multi-environment advanced yield and elite trials over 8 years (2014–2021). Phenotyping for WAC was done via the solvent retention capacity (SRC) using water as a solvent (SRC-W). Traits that exhibited a significant correlation ($r \geq 0.3$) with SRC-W and were evaluated earlier than SRC-W were included in the multivariate genomic prediction models. Kernel hardness and diameter were obtained using the single kernel characterization system (SKCS), and break flour yield and total flour yield (T-Flour) were included. Cross-validation showed the mean univariate genomic prediction accuracy of SRC to be $r = 0.69 \pm 0.005$, while bivariate and multivariate models showed an improved prediction accuracy of $r = 0.82 \pm 0.003$. Forward validation showed a prediction accuracy up to $r = 0.81$ for a multivariate model that included SRC-W + All traits (SRC-W, Diameter, SKCS hardness and diameter, F-Flour, and T-Flour). These results suggest that incorporating correlated traits into genomic prediction models can improve early-generation prediction accuracy.

1 | INTRODUCTION

The primary objective of wheat breeding programs is to improve grain yield to meet the increasing demand for wheat (*Triticum aestivum* L.) due to rapid population growth (Alahmad et al., 2022; Xiong et al., 2008). Population growth not only affects wheat quantity but also changes food preferences

Abbreviations: B-Flour, break flour yield; BLUE, best linear unbiased estimate; CSU, Colorado State University; GBS, genotyping-by-sequencing; GEBV, genomic estimated breeding value; SNP, single-nucleotide polymorphism; SRC, solvent retention capacity; SRC-W, solvent retention capacity using water as a solvent; T-Flour, total flour yield; WAC, water absorption capacity.

and consumption trends (Schneider et al., 2011; Xiong et al., 2008), driving the need to improve the milling, dough mixing, and baking quality required for a variety of wheat-based foods like bread, noodles, crackers, cookies, pizza, and cereal bars.

The differences between hard and soft wheat classes are based on kernel texture, milling quality, protein strength, and water absorption (Kiszonas et al., 2013; Souza et al., 2002). Hard wheat is favored over soft wheat for bread and pizza doughs due to its higher protein concentration, higher water absorption, and stronger gluten (Katyal et al., 2017; Wieser, 2007). Water absorption capacity (WAC) influences dough characteristics such as dough extensibility, crumb texture, loaf volume (Zghal et al., 2001), bread yield (Puhr & D'Appolonia, 1992), and shelf life (Pyler, 1979). To optimize flour's WAC, one can use flour from a variety with a higher absorption capacity or add extracted gluten, though the latter is not cost-efficient. A variety with a higher WAC is preferable for its sustainability and cost-effectiveness. Developing such a variety is challenging, as important quantitative traits like grain yield and quality are influenced by environmental factors, such as weather conditions, soil fertility, and disease and insect pests, as indicated by Bilsborrow et al. (2013) and Hua et al. (2022).

The short time between harvesting and planting in winter wheat breeding programs often limits opportunities to collect WAC and other end-use quality data in a timely manner (Nelson et al., 2016). Phenotyping quality traits is time- and labor-intensive and may require larger grain samples that are often unavailable during early-stage line development (Jernigan et al., 2018; Kiszonas et al., 2013; Sandhu et al., 2021). Consequently, selection for quality traits is often delayed, leading to advanced breeding lines or released varieties with desirable yields but suboptimal quality (Bassi et al., 2016; Jernigan et al., 2018).

Genomic prediction is an indirect selection approach that enhances the accuracy of marker-assisted selection by using genome-wide markers to capture quantitative trait loci with small to large effects (Bernardo, 2002; Hayes et al., 2009; Larkin et al., 2019; Meuwissen et al., 2001). It offers several key advantages over phenotypic selection, including early selection, enhanced accuracy through genomic data integration, and selection for traits that are difficult to measure. One of the critical improvements genomic prediction brings is in reducing cycle time and cost, particularly relevant in quality traits where delayed parental selection until quality data are available has traditionally been a bottleneck. By enabling earlier selection decisions, genomic prediction overcomes this limitation and increases the rate of genetic gain. Additionally, it provides more accurate predictions of genetic potential by leveraging genomic information. Lastly, genomic prediction enhances selection efficiency by considering multiple traits and markers, which leads to a more efficient and precise selection of genotypes with desirable quality traits (Shahi et al., 2022).

> **Core Ideas**
> - Genomic prediction can be used to improve end-use quality traits like water absorption capacity in hard winter wheat.
> - Correlated traits enhance prediction accuracy when included in a multivariate prediction model.
> - Multivariate models excel in predicting water absorption capacity compared to univariate models.

The prediction accuracy of genomic prediction can be influenced by factors such as training population size, marker density, trait heritability, population structure, and relationship of the training population to selection candidates (Bassi et al., 2016; Larkin et al., 2019; Robertson et al., 2019). Various approaches have been proposed to improve prediction accuracies, including reducing confounding factors like population structure, increasing training population size, and selecting appropriate models (Larkin et al., 2019; Sallam et al., 2020). These models may incorporate marker-trait association scores from genome-wide association studies (Medina et al., 2021; Zhang et al., 2019), machine learning models (Jubair & Domaratzki, 2023; Zhang et al., 2019), multi-environment models (Guo et al., 2020; Tomar et al., 2021), and multivariate genomic prediction models that include correlated traits (Atanda et al., 2022; Guo et al., 2020; Montesinos-López et al., 2021; Winn et al., 2023). Several studies have applied multivariate genomic prediction methods to quality traits (Azizinia et al., 2022; Ibba et al., 2020; Montesinos-López et al., 2021; Sandhu et al., 2021). While several studies (Battenfield et al., 2016; Guo et al., 2020; Sandhu et al., 2021) have reported genomic predictions for WAC using solvent retention capacity test using water as a solvent (SRC-W) for soft white winter wheat, literature focusing on genomic prediction of WAC and SRC-W in hard winter wheat appears to be lacking.

Forward prediction, which predicts future generations using previous generations' data as the training population, is often employed in breeding programs (Belamkar et al., 2018; Calvert et al., 2020; Jarquín et al., 2017). Forward prediction assesses the performance of genomic predictions for lines and environments not yet phenotyped (Haikka et al., 2020). Although previous studies have aimed to improve prediction accuracy by adjusting (filtering, cleaning, and using more balanced data) and cross-validating training populations (Combs & Bernardo, 2013; Hayes et al., 2009; Larkin et al., 2019), the true effectiveness of GS lies in its practical application in breeding programs and validating the performance of genomic predictions.

WAC in hard winter wheat is an important breeding target. Despite the complex and time-consuming nature of phenotyping WAC, the use of genomic tools can efficiently enhance selection for WAC in generations where phenotyping is impractical or infeasible. To assess the potential of genomic prediction of WAC, the following objectives were addressed in the current study: (1) explore the association between WAC, flour yield traits, and early generation-applicable traits from the single kernel characterization system (SKCS) to determine their potential for inclusion in multivariate genomic prediction models; (2) assess the accuracy of univariate and multivariate genomic prediction for WAC through cross-validation; and (3) determine the accuracy of genomic prediction in a breeding scenario through forward validation.

2 | MATERIALS AND METHODS

2.1 | Germplasm

A total of 497 hard winter wheat genotypes (experimental lines and check varieties) generated from the Colorado State University (CSU) wheat breeding program were utilized in this study. These genotypes were evaluated within their respective breeding cycles under different independent trials, including the CSU Elite Trial, the advanced yield nursery (AYN), and the advanced yield nursery composed of doubled haploid lines (AYND). For a given nursery-year combination, genotypes phenotyped for quality traits may have been sampled from varying numbers of sites. A summary of the number of genotypes and observations in each nursery from 2014 to 2021 is provided (Table 1).

2.2 | Experimental design and trait measurements

The CSU Elite Trials were organized using resolvable, latinized row-column designs with partial replication, as per the methodologies outlined by John and Williams (1995) and Williams et al. (2011). Within the CSU Elite Trials, at a given site, half of the entries were replicated twice, and half were replicated once. In the AYNs, at a given site, roughly one-seventh of the entries were replicated twice, and the remaining entries were replicated once. All genotypes included in this study were evaluated at CSU wheat breeding program sites located in the Great Plains wheat-growing region of eastern Colorado. All trials were conducted "on farm" in grower cooperators' fields, except for those at the Agricultural Research, Development, and Educational Center in Fort Collins, CO, Plainsman Research Center in Walsh, CO, and the United States Department of Agriculture–Agriculture Research Service Central Great Plains Center in

TABLE 1 Number of observations and genotypes used in this study per year per trial.

Year	Trial	No. of sites	No. of observations	No. of genotypes
2014	AYND[a]	3	105	35
2015	AYN[b]	2	56	29
	ELITE[c]	2	68	34
2016	AYN	3	99	33
	ELITE	5	165	33
2017	ELITE	4	140	35
2018	AYN	3	90	30
	ELITE	5	95	19
2019	AYN	3	75	25
	AYND	3	78	35
	ELITE	6	185	37
2020	AYN	3	93	31
	AYND	3	96	33
	ELITE	4	116	29
2021	AYN	2	52	26
	ELITE	3	99	33

[a]Advanced doubled haploid yield nursery.
[b]Advanced yield nursery.
[c]CSU Elite Trial.

Akron, CO. The agronomic and crop management practices mirrored those adopted by the grower cooperators and were adjusted based on the standard practices at each site. Each plot measured 1.5 m wide and 3.7 m long and was planted in six rows. The seeding rate was approximately 1.73 million seeds ha^{-1}. All six rows of each plot were harvested, and a cleaned sample of the grain was used for subsequent quality analyses.

In this study, traits included as covariates were chosen based on their phenotypic correlation with values from the solvent retention capacity (SRC) test using water as a solvent (SRC-W) and their timing in the data collection process relative to SRC-W. The phenotypic correlations are presented in Figure S1. Traits that would normally be evaluated after SRC-W in the overall testing scheme or traits showing a correlation of $r \leq [0.3]$ were excluded: grain protein concentration, bake water absorption, bake mixing time, bake loaf volume, Mixograph mixing tolerance, Mixograph mixing time, Mixograph midline left slope, Mixograph midline right slope, Mixograph midline peak value, Mixograph midline peak width, and Mixograph midline right width. Traits utilized for subsequent analyses include SKCS kernel hardness and kernel diameter, break flour yield (B-Flour), and total flour yield (T-Flour). The phenotyping details are presented only for the selected traits.

All traits included in this study were analyzed in the CSU Wheat Quality Lab located in Fort Collins, CO, following the procedures of the American Association of Cereal

Chemists International (AACC International, 2010). SKCS kernel diameter and kernel hardness were evaluated using 10–15 g of grain using the Single Kernel Characterization System 4100 (Perten Instruments, Springfield, IL; AACC-approved method 55-31.01). Grain samples (50 g) for each genotype were tempered to 15 g kg^{-1} (15%) moisture and milled using a modified Brabender Quadrumat Senior Mill (Brabender Instruments). The material passing the first break roll was separated by a two-stage sieve where only the first break-flour passes through the sieve. After measuring the first break flour, the remaining material underwent further milling over the reduction rolls. The T-Flour was measured (in g kg^{-1}) as the combined total of white flour from the first and second mill streams. The B-Flour measured (in g kg^{-1}) of the white flour from the first pass through the mill rolls.

WAC was determined from white flour samples using the SRC test using water as a solvent according to AACC-approved method 56-11.02. Empty 2 mL microcentrifuge tubes were labeled and weighed for each sample. Each tube was then filled with 200 mg of flour for each sample. Tubes were mixed with 1 mL double distilled water and vortexed for 5 s to suspend the flour. The mixture was then shaken for 20 min using a rotator, ensuring thorough mixing of the flour and water. Subsequently, samples were centrifuged for 15 min at 1000 g at room temperature. After centrifugation, the supernatant was removed, and the gel-containing tubes were dried for 10 min and then weighed. The gel (pellet) weight was calculated by subtracting the weight of the empty tube from the weight of the tube with the pellet. The SRC value for each sample was then calculated according to the following formula:

$$\text{SRC (\%)} = \left[\frac{\text{Pellet weight} (g)}{\text{Flour weight} (g)} - 1 \right] \times \left[\frac{(86)}{100 - \text{moisture} (\%)} \right] \times 100$$

where SRC (%) is the SRC in percentage, and moisture (%) is the flour moisture determined using a Foss DA1650 near-infrared spectrometer (Foss North America).

2.3 | Phenotypic data analysis

Across trial-site-year best linear unbiased estimates (BLUEs) were calculated using the *ASREML-R* package in R statistical software (R Core Team, 2013). The following mixed linear model was used to estimate BLUEs across trial-site-year combinations:

$$Y = \mu + G + E : R + E : C + \varepsilon$$

where Y is the vector of the observed values, G is the fixed genotypic effect, $E:R$ is the random row effect nested environ-

ment (trial-site-year combination), $E:C$ is the random column effect nested into environment, and the ε is the vector of residuals.

Summary statistics (mean, minimum, maximum, and standard deviation) were calculated to assess the distribution of the data across the range of values in the population. Pearson correlation coefficients were calculated and visualized using the *psych* package in R (Revelle & Revelle, 2015).

A multivariate mixed linear model was performed to calculate the genetic correlations and variance components to estimate heritability for all traits included in this study. The following model was used:

$$\begin{bmatrix} Y_1 \\ \dots \\ Y_k \end{bmatrix} = X \begin{bmatrix} \beta_1 \\ \dots \\ \beta_k \end{bmatrix} + Z \begin{bmatrix} u_1 \\ \dots \\ u_k \end{bmatrix} + \varepsilon$$

where Y_k is the $n \times 1$ matrix of observed values (BLUEs) of kth trait, β_k is the fixed effect for the kth trait, X is the design matrix for the fixed effects, u_k denotes the random effects associated with the kth trait, Z is the design matrix for the random effects, and ε is the vector of residual errors.

Genetic correlation among traits was calculated as:

$$r_g = \frac{\sigma^2_{y_1, y_2}}{\sqrt{\sigma^2_{y_1} \times \sigma^2_{y_2}}}$$

where $\sigma^2_{y_1, y_2}$ is the covariance between two traits, $\sigma^2_{y_1}$ is the variance of the first trait, and $\sigma^2_{y_2}$ is the variance of the second trait.

Entry mean genomic heritability was calculated as follows:

$$h^2_g = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_\varepsilon}$$

where σ^2_g is the genetic variance and σ^2_ε is the residual error.

2.4 | Genotyping

Genotyping was done using genotyping-by-sequencing (GBS). Genomic DNA was extracted from 1-week-old wheat leaves in a 96-well format using the MagMax14 plant DNA kit and quantified using PicoGreen (Thermo Fisher Scientific) assays. For GBS library construction, a modified protocol from Poland et al. (2012) was used to create libraries (384-plex), which were sequenced on an Illumina HiSeq2500 platform at the University of Illinois. Single-nucleotide polymorphism (SNP) calling was performed using the *TASSEL 5.0* GBSv2 pipeline (Glaubitz et al., 2014), with a 64-base k-mer length and a minimum k-mer count of five. Sequences were aligned to the International Wheat Genome Sequencing

Consortium RefSeq v2.0 Chinese Spring wheat reference sequence (Appels et al., 2018) using the Burrows-Wheeler aligner version 0.7.10 (Li & Durbin, 2009).

The raw SNP data generated from the *TASSEL* pipeline were filtered to remove individuals with >50% missing data and average heterozygosity >30%. Markers were filtered to select biallelic SNPs with a minor allelic frequency >5%, <10% missing data, and average heterozygosity less than or equal to 10%. Missing GBS data were imputed using the Beagle algorithm (Browning et al., 2018). All unaligned SNPs were dropped from the dataset prior to imputation. After filtering and imputation, 23,130 SNPs were used for subsequent analyses.

2.5 | Univariate and multivariate genomic prediction

Univariate genomic prediction for SRC-W was performed using a genomic best linear unbiased prediction model using *ASREML* function with *ASREML-R* package as follows:

$$y = X\beta + Zu + \varepsilon_i$$

Where u represents the vector of genotype effects, assumed to follow a normal distribution $u \sim N(0, G\sigma_u^2)$, with G being the genomic relationship matrix, which is calculated using the *GRM* function from the *gaston* package in R (Perdry & Dandine-Roulland, 2023) and σ_u^2 is the variance of individual genotype effects; β is the vector of fixed effects (the overall mean); and X and Z are the design matrices associated with fixed and random effects, respectively. The design matrix Z has m markers in its columns and no observed genotypes in its rows. The residual error represented by ε_i, is assumed to have a normal distribution $\varepsilon_i \sim N(0, I\sigma_\varepsilon^2)$, where I is the identity matrix, and σ_ε^2 is the residual error variance. The genomic estimated breeding value (GEBV) was calculated as the sum of the additive allele effects for a given genotype (Chen & Zhang, 2018; VanRaden, 2008).

In the multivariate genomic prediction, the same model as univariate genomic prediction was used.

$$\begin{bmatrix} Y_1 \\ \dots \\ Y_k \end{bmatrix} = X \begin{bmatrix} \beta_1 \\ \dots \\ \beta_k \end{bmatrix} + Z \begin{bmatrix} u_1 \\ \dots \\ u_k \end{bmatrix} + \varepsilon$$

However, the random effect is described as follows:

$$\begin{bmatrix} u_1 \\ \dots \\ u_k \end{bmatrix} \sim N(\begin{bmatrix} 0 \\ \dots \\ 0 \end{bmatrix}, \begin{bmatrix} \sigma_{11}^2 & \sigma_{12}^2 & \dots & \sigma_{1k}^2 \\ \dots & \dots & & \dots \\ \sigma_{k1}^2 & \sigma_{k2}^2 & \dots & \sigma_{kk}^2 \end{bmatrix} \otimes G), \text{where } \otimes \text{ is the}$$

Kronecker product and G is the genomic relationship matrix, and σ_{ij}^2 is the variance or covariance between ith and jth trait. The residual ε uses the unstructured variance matrix

as defined by software *ASREM-R*. In the univariate genomic prediction, $k = 1$, while in the multivariate genomic prediction, $k > 1$. The GEBVs were calculated for each trait as the cumulative sum of the additive allele effects for a specific genotype.

The accuracy of genomic prediction was assessed through both cross-validation and forward validation. In cross-validation, the dataset was randomly split into training (80%) and testing (20%) sets, and the model was trained to predict GEBVs for the testing set. This process was repeated 100 times with different random subsets of the data for training and testing to evaluate the prediction accuracy of the model. Prediction accuracy was considered as the Pearson correlation coefficient (r) between the GEBVs and the observed data (BLUEs). The prediction accuracy of all models was visualized using a box plot constructed with the *ggplot2* package in R (Wickham, 2011).

The forward validation process involved the same 497 genotypes that were part of the cross-validation between 2014 and 2021. Seven different validation sets were formed using the CSU Elite Trial data from 2019 to 2021. First, the BLUEs from the Elite trial were used as a validation set (FV_2019, FV_2020, and FV_2021). Subsequently, the data from each year with another year were combined to create 2-year forward validation sets, designated as FV_2019&2020, FV_2019&2021, and FV_2020&2021. Finally, the data from all 3 years were assigned as a validation set (FV_2019–2021). The data remaining after the exclusion of the validation set were utilized as the training set for the corresponding evaluation. Unlike cross-validation, the entire prediction process in forward validation is non-iterated, making it a fixed, one-time assessment based on the initial data split. The number of observations for each genotype each year is described (Table 1).

In both cross-validation and forward-validation multivariate genomic prediction, only the data for SRC-W in the validation set were masked for prediction in the training set. However, the data for the correlated traits of these genotypes remained in the model. For instance, during forward prediction model training, the SRC-W data for 58 genotypes from 3 years of CSU Elite Trials (2019–2021) were masked, while the covariate trait values associated with these genotypes were included in the models.

3 | RESULTS

3.1 | Phenotypic variation

Summary statistics demonstrate that there is considerable genotypic variation for each trait (Table 2). SKCS kernel hardness displayed the greatest range between its minimum (49.2) and maximum values (86.6), indicating a wider variance in

TABLE 2 Summary statistics for quality traits included in this study.

Traits	Mean	Min	Max	SD	h^2	SE h^2
SRC-W	60.6	54.4	71.1	2.75	0.58	0.09
SKCS kernel diameter	2.6	2.3	2.9	0.09	0.50	0.09
SKCS kernel hardness	68.7	49.2	86.6	6.14	0.57	0.09
B-Flour	47.5	34.5	54.1	3.06	0.59	0.08
T-Flour	69.3	62.1	74.3	1.76	0.55	0.08

Note: SRC-W refers to the solvent retention capacity using water as a solvent, SKCS refers to a single kernel characterization system, B-Flour refers to break flour yield, and T-Flour refers to total flour yield.

Abbreviations: B-Flour, break flour yield; h^2, heritability; Max, maximum; Min, minimum; Sd, standard deviation; SE h^2, standard error of the heritability; T-Flour, total flour yield.

the total population for this trait. The mean SRC-W was 60.6%, with a minimum of 54.4% and a maximum of 71.1%. Traits exhibited moderate heritability values, ranging from 0.50 ± 0.09 to 0.59 ± 0.08 (Table 2). The trait with the highest heritability was B-Flour ($h^2 = 0.59 \pm 0.08$), with a mean of 47.5 g kg^{-1}, followed by SRC-W with ($h^2 = 0.58 \pm 0.09$). The lowest heritability $h^2 = 0.50 \pm 0.08$ was observed for SKCS kernel diameter.

The Pearson correlation results showed both strong positive and negative correlations between the variables. The strongest positive correlation ($r = 0.82$, $p < 0.001$) was observed between B-Flour and T-Flour, whereas the strongest negative correlation ($r = -0.75$, $p < 0.001$) was observed between SRC-W and B-Flour (Figure 1a). Similarly, T-Flour showed a significant negative correlation ($r = -0.54$, $p < 0.001$) with SRC-W. SKCS kernel hardness showed a significant positive correlation ($r = 0.61$, $p < 0.001$) with SRC-W and a significant negative correlation with B-Flour ($r = -0.68$, $p < 0.001$) and T-Flour ($r = -0.46$, $p < 0.001$). However, no correlation ($r = 0.00$, $p > 0.05$) was observed between SKCS kernel diameter and T-Flour, and a very weak correlation ($r = 0.06$, $p > 0.05$) was observed between SKCS kernel hardness and SKCS kernel diameter.

Genetic correlation values ranged from $r = -0.76$, $p < 0.001$, to $r = 0.74$, $p < 0.001$ (Figure 1b). There were observable differences compared to the phenotypic correlations, while some changed in magnitude. For instance, the phenotypic correlation between SKCS kernel hardness and T-Flour ($r = -0.46$, $p < 0.001$) was decreased in the genetic correlation ($r = -0.21$, $p < 0.05$). The same trend was shown for the genetic correlation between SRC-W and T-Flour, which reduced from ($r = -0.54$, $p < 0.001$) for the phenotypic correlation to $r = -0.42$, $p < 0.01$ for the genetic correlation. However, no change in the direction of the correlation was observed.

## 3.2 | Univariate versus multivariate genomic selection cross-validated accuracies

The prediction accuracies of the univariate, bivariate, and full multivariate models for predicting SRC-W were validated using a cross-validation approach. These models exhibited varying degrees of accuracy, ranging from $r = 0.69 \pm 0.005$ to $r = 0.82 \pm 0.003$ (Figure 2). The analysis encompassed a progression from a univariate model to four bivariate models, concluding with a full multivariate model. The univariate model, utilizing only SRC-W as a predictor, showed a prediction accuracy of $r = 0.69 \pm 0.005$. When SKCS kernel diameter was included in the first bivariate model, there was a marginal improvement in the prediction accuracy to $r = 0.70 \pm 0.004$, representing only a one percent increase over the univariate model.

A higher prediction accuracy of $r = 0.78 \pm 0.004$ was observed in the second bivariate model that included SKCS kernel hardness. This model demonstrated a notable 9% improvement over the univariate model. Regarding the two other bivariate models that integrated milling-related traits, prediction accuracy with the inclusion of T-Flour was $r = 0.75 \pm 0.004$ and $r = 0.82 \pm 0.003$ with the inclusion of B-Flour. The multivariate model, which combined all grain and flour-related traits, showed a prediction accuracy of $r = 0.82 \pm 0.003$, identical to the highest accuracy observed with the bivariate model incorporating B-Flour. This represents a 13% prediction accuracy improvement over the univariate model, making it the highest accuracy observed among the six models.

3.3 | Forward validation accuracies in the CSU Elite Trials

Forward validation was also done to assess the accuracy of the univariate, bivariate, and multivariate models. Data were partitioned into 1-year, 2-year, and 3-year forward validation sets. In the 3-year validation set (FV_2019-2021), the prediction accuracy ranged from $r = 0.65$ for the univariate model to $r = 0.81$ for the SRC-W + B-Flour bivariate model and a model including all measured traits (Figure 3). The bivariate model, including SRC-W and SKCS kernel hardness, had a prediction accuracy of $r = 0.75$, marking a 10% improvement compared to the univariate model from the FV_2019-2021 set. On the other hand, the inclusion of SKCS kernel diameter exhibited very little improvement in prediction accuracy ($r = 0.66$), only a one percent improvement from the univariate model.

Among all validation sets, the single-year set (FV_2019) exhibited the highest multivariate prediction accuracy ($r = 0.93$), as indicated by the multivariate model (Figure S2, which represents a 12% increase over the highest prediction
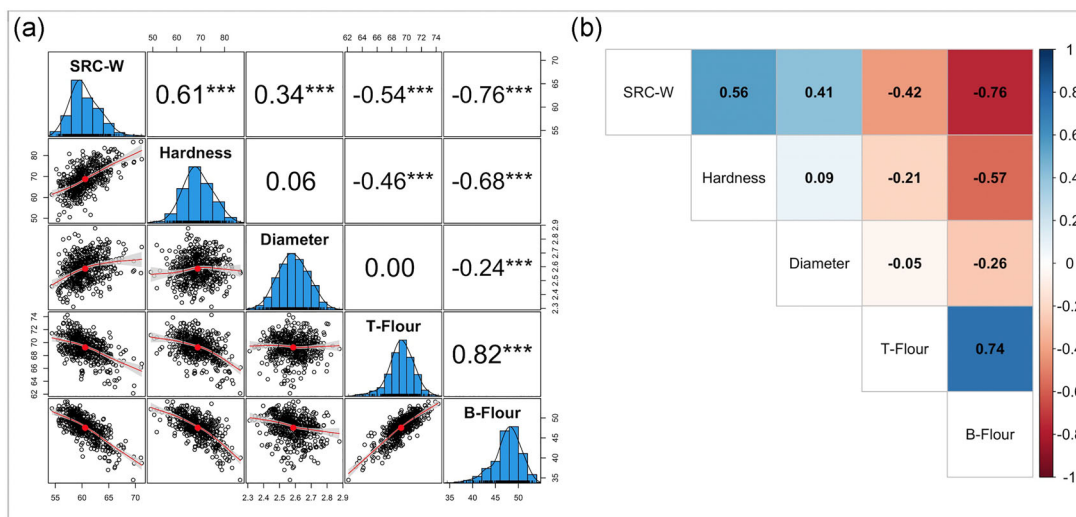
FIGURE 1 Phenotypic and genetic correlation among measured traits. (a) Pearson's correlation coefficient plot in which histograms and trait names are displayed on the central diagonal. The traits represented include solvent retention capacity using water as a solvent (SRC-W), single kernel characterization system (SKCS), SKCS kernel diameter (Diameter), SKCS kernel hardness (Hardness), break flour yield (B-flour), and total flour yield (T-Flour). The scatterplots of the traits and smoothed regression lines are shown in the lower half of the boxes. The values on the upper half of the diagonal represent the correlation coefficients between the traits. The significance values of the correlation for corresponding traits were as follows: ns = non-significant, $*$ = 0.05, $**$ = 0.01, and $***$ = 0.001. If no stars are present, the correlation of the variables indicated by the boxes is not significant. (b) Heatmap correlation plot, which illustrates the pairwise genetic correlations between variables in the dataset. Each cell in the grid represents the genetic correlation coefficient between two variables, with color intensity indicating the strength and direction of the correlation. The darker blue colors indicate stronger positive correlations, the darker red colors indicate stronger negative correlations, and lighter colors indicate weaker or no correlation.
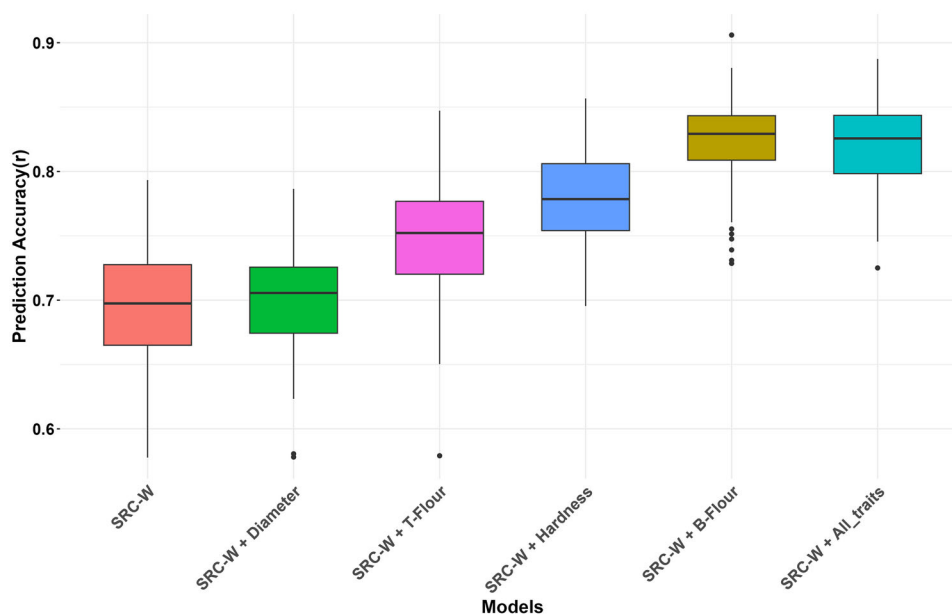
FIGURE 2 Cross-validation prediction accuracy (r) of six different genomic selection models. The x-axis displays the model type with the trait name, such as solvent retention capacity using water as a solvent (SRC-W), single kernel characterization system (SKCS) kernel diameter (Diameter) and kernel hardness (Hardness), total flour yield (T-Flour), break flour yield (B-Flour), and all traits when all covariates were included in the model. The "+" symbol indicates that multiple traits are included in the model. The y-axis represents the correlation coefficient (r) between the genomic estimated breeding value and the best linear unbiased estimated value. Each box represents the interquartile range of the distribution of correlation coefficients, with the median indicated by a horizontal line within the box. Data points outside the box are considered outliers.
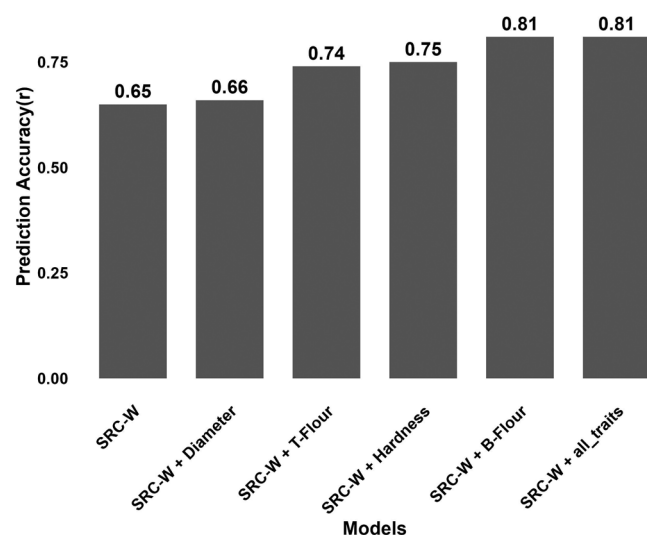
FIGURE 3 Bar charts of forward-validated prediction accuracies for solvent retention capacity using water as a solvent (SRC-W) derived from 3 years of Colorado State University (CSU) Elite Trials conducted by the Colorado State University Wheat Breeding Program. The prediction accuracy represented as Pearson's correlation coefficient (*r*) is shown on the *y*-axis and is also explicitly displayed on top of the corresponding bar. The *x*-axis shows the names of the respective models tested—one univariate, four bivariate, and one comprehensive multivariate model, in that order. Covariates are included in the models with Diameter (SKCS kernel diameter), Hardness (SKCS kernel hardness), B-flour (break flour yield), and T-Flour (total flour yield).

accuracy observed from the 3-year validation set (FV_2019-2021). Conversely, the lowest prediction accuracy range was found for the FV_2021 set, with values ranging from $r = 0.44$ (for the univariate SRC-W model) to $r = 0.61$ (for the multivariate SRC-W + All traits model) model (Figure S2). This marks a 20% decrease from the highest prediction accuracy observed for the FV_2019-2021 set and a substantial 32% decrease compared to the FV_2019 single-year validation set. Generally, all sets, including various combinations from the FV_2021, exhibited lower accuracies than those excluding FV_2021. The FV_2021 set comprised 33 genotypes, while the FV_2020 and FV_2019 sets had 29 and 37 genotypes, respectively. Notably, the FV_2019 set outperformed others in prediction accuracy, with sets integrating 2019 CSU Elite Trial data showing modest improvements.

4 | DISSCUSSION

In the current study, a historical dataset of hard winter wheat quality data from multiple sites and years was analyzed together with their genotypic data. Univariate and multivariate models were compared in terms of their prediction accuracy for WAC as measured using the SRC test with water as a solvent (SRC-W). Both cross- and forward-validation approaches were employed to validate the robustness and predictive accuracy of the models tested. Generally, for both cross-validation and forward prediction, the accuracy of predictions improved in multivariate models when the covariate demonstrated higher or nearly identical heritability and showed a significant correlation with the target trait. Traits used as covariates in this study include SKCS kernel hardness and diameter, B-flour, and T-Flour.

The moderate heritability observed for all traits included in this study suggests a balance between genetic and environmental influences on their expression. This balance implies the potential for effective selection while also underscoring the role of environmental or management interventions in affecting their expression (Bernardo, 2002; Holland et al., 2003). Traits such as B-flour, T-Flour, and SKCS kernel hardness showed almost the same heritability and a strong positive correlation with SRC-W, and when included in the models, they led to improved prediction accuracies. However, the SKCS kernel diameter showed a weak correlation with SRC-W, and its inclusion as a covariate did not result in an appreciable improvement in prediction accuracy. This indicates that strong correlations and heritability are the main factors in improving prediction accuracy in multivariate models, in agreement with previous studies (Crossa et al., 2017; Ibba et al., 2020).

The multivariate (SRC-W + All traits) and bivariate (SRC-W + B-Flour) models showed a 13% increase in prediction accuracy compared to the univariate and the other bivariate models. These results underscore the significance of multivariate models over univariate models and indicate that these two models were the best fit for the data, successfully capturing the underlying genetic variation. Similarly, the bivariate model that included SRC-W and SKCS kernel hardness showed increased prediction accuracy. Here's how these three traits interplay: harder grains require greater milling force, which leads to increased starch damage, which in turn increases the WAC during dough mixing (Arya et al., 2015; Kweon et al., 2011; Sapirstein et al., 2018). On the other hand, B-Flour is negatively correlated with grain SKCS kernel hardness, as harder grains produce less break flour, and thus more of the total product must pass over the reduction rolls, leading to greater starch damage and thus increased water absorption (Hogg et al., 2004; Symes, 1969). This suggests that both B-Flour and SKCS kernel hardness are critical traits that, in combination, influence the WAC of the milled flour and, by extension, the predictive accuracy of the models.

Previous studies have shown the effectiveness of multivariate models over univariate models. Gill et al. (2023) in winter wheat and Bhatta et al. (2020) in barley (*Hordeum vulgare* L.) found that a multivariate model outperformed a univariate model in predicting grain yield using agronomic traits as covariates. Similarly, Montesinos-López et al. (2021) reported improved prediction accuracy for grain yield with

multivariate models using quality traits as covariates compared to univariate models with no covariates. Moreover, multivariate models outperformed univariate models for predicting grain yield and quality traits in wheat (Guo et al., 2020; Lozada & Carter, 2020; Sandhu et al., 2021). These findings support the advantages of utilizing multivariate models for predicting wheat quality traits, leading to improved prediction accuracy compared to univariate models.

Incorporating a correlated trait in multivariate models may not always result in improved prediction accuracy, especially when the covariate has low heritability, and the correlation is not strong (Shahi et al., 2022; Sun et al., 2017). The correlation between SKCS kernel diameter and SRC-W was significant but relatively lower in magnitude, resulting in no improvement in prediction accuracy compared to the univariate model. This might be due to environmental variation or interactions that weren't captured by the respective models or due to the traits not being correlated (Sandhu et al., 2022). Other reports have also shown no improvement in certain multivariate models, including Lado et al. (2018) for wheat baking quality traits and Schulthess et al. (2016) for grain yield and protein concentration in rye (*Secale cereale* L.).

In this study, forward validation accuracy was similar to that observed for cross-validation (less than a five percent difference between the two validation approaches). Indicating that the models would tend to be useful in a breeding program for predicting genotypes that were not part of the training panel (unseen data) (Battenfield et al., 2016; Yadav et al., 2021). Such performance is a testament to a robust model that is applicable beyond its training data (Yadav et al., 2021). The similar prediction accuracy between the two validation methods further emphasizes the model's consistent performance, regardless of the validation approach used.

In most cases, achieving higher prediction accuracy for forward validation is challenging, as it involves predicting future outcomes where environmental conditions, management practices, and other factors may differ from the data used in the training set (Hoffstetter et al., 2016; Jarquín et al., 2017; Juliana et al., 2018; Lozada & Carter, 2020). Several possible reasons account for the higher prediction accuracy observed in this study using both validation approaches. One reason is that the genotypes used in both the validation and training sets were sourced from the same breeding program, suggesting a high probability of a shared genetic background. This similarity in genetic makeup can increase the representativeness of the training set, potentially leading to improved prediction accuracy (Battenfield et al., 2016). Another reason might be the quality of the phenotypic data used, which can significantly affect prediction accuracy. Accurate phenotypic data are a primary driving factor for increased prediction accuracy and genetic gain, and genomic prediction, in turn, relies on the quality of this phenotypic data (Bartholomé et al., 2022; Beyene et al., 2019; Sandhu et al., 2021). Another common concern is overfitting, where a model performs well on training data but underperforms with new data (Montesinos López et al., 2022). However, the similar accuracies observed in this study for both cross-validation and forward validation mitigate these concerns. In essence, the observed higher prediction accuracies suggest that the models are reliable, robust, and well-suited for generalizing to new individuals.

The comparable prediction accuracy results observed with both validation approaches contrast with previous reports of higher prediction accuracy from forward validation over cross-validation. For instance, Zhang et al. (2022) reported higher prediction accuracy with forward validation for Fusarium head blight resistance in hard winter wheat. Similarly, Haikka et al. (2020) observed a slight increase in prediction accuracy using forward validation in their genomic study of Finnish oats (*Avena sativa* L.) and barley. Additionally, Azizinia et al. (2023) reported improved forward prediction accuracy after incorporating extra years into their multi-trait wheat quality model. Furthermore, Fradgley et al. (2023) in wheat and Zystro et al. (2021) in sweet corn (*Zea mays* L.) also observed higher forward prediction accuracy than with a cross-validation approach.

However, several authors have reported lower prediction accuracies with forward validation. Jarquín et al. (2017) found lower prediction accuracy (by 37%) for grain yield in wheat using a forward validation approach. Similarly, Battenfield et al. (2016) reported a 19% decrease in forward validation over the cross-validation, and Sweeney et al. (2019) also reported reduced prediction accuracy (by 35%) from forward validation for wheat end-use quality traits. Dawson et al. (2013) observed lower prediction accuracy (lower by 17%) from a forward validation approach for wheat grain yield.

5 | CONCLUSIONS

The current work has demonstrated the strong potential of multivariate genomic prediction for improving WAC in hard winter wheat. The utilization of forward and cross-validation methods in this study has shown their effectiveness in achieving high predictive accuracies. By including easily obtainable covariates in genomic prediction, the accuracy of SRC-W prediction was significantly improved, highlighting the importance of carefully selecting traits when constructing prediction models. Although incorporating flour yield values as covariates may pose challenges due to time consuming and costly phenotyping processes, the SRC-W + SKCS kernel hardness model, presented in this study offers a practical and cost-effective solution. The comparable results with minimal differences between the two validation approaches in this study suggest that the utilization of high-quality phenotypic data and the genetic relatedness within the breeding material can notably enhance the predictability of forward

validation, potentially aligning it more closely with the outcomes of cross-validation. Overall, these findings underscore the use of genomic prediction in improving WAC, demonstrating its potential for early-stage selection of high-performing genotypes and advancing the breeding process.

AUTHOR CONTRIBUTIONS

Meseret Wondifraw: Conceptualization; data curation; formal analysis; methodology; software; visualization; writing—original draft; writing—review and editing. **Zachary J. Winn**: Data curation; formal analysis; writing—review and editing. **Scott D. Haley**: Conceptualization; data curation; methodology; project administration; resources; supervision; writing—review and editing. **John A. Stromberger**: Data curation; resources. **Emily Hudson-Arns**: Conceptualization; funding acquisition; project administration; supervision; writing—review and editing. **R. Esten Mason**: Conceptualization; funding acquisition; project administration; resources; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The phenotypic and genotypic data supporting this study are available in DRYAD at: https://datadryad.org/stash/share/h_4eJT85wfZKwku0_8yStKYBcrUzfgBb3Z2AJx4MfQo, https://doi.org/10.5061/dryad.6wwpzgn71.

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