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The use of fixed shelling percentage biases genotype selection in hybrid maize multi-environment yield trials

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ABSTRACT

Context or problem: Phenotyping is an integral part of plant breeding operations. In many cases the trait measured is not identical to the target trait for reasons of speed and or cost. This is a form of indirect selection, where correlation between the trait measured and the target phenotype influences the rate of genetic gain. Low correlations lead to slow rates of genetic gain. In sub-Saharan African maize breeding programs, maize grain yield in breeding experimental plots is measured as a field weight (FW), which includes the grain and cob. The weight of grain from each plot is estimated as a standard proportion of grain to total ear weight using a shelling percentage of 80 %. This approach assumes that there is no genetic, environment or genetic by environment interaction in shelling percentage which, if present, would contribute to slower rates of genetic gain for grain yield.
Objective or research question: This study investigated the magnitude of genetic and environmental variation in shelling percentage and its impact on selection in six hybrid maize multi-environment yield trials in Ethiopia over two seasons.
Methods: The data of shelled grain weight (SW) and cob weight (CW) from the trials were analyzed using a bivariate linear mixed model.
Results: Genetic variances for both traits varied across the six testing sites ranging from 0.199 to 2.975 for SW and from 0.029 to 0.245 for CW. The genetic correlations between pairs of sites for SW and CW also varied, indicating the existence of genotype by environment interaction for these traits. Additionally, the bivariate regressions between FW and SW indicated there was substantial genetic deviation around the 80 % shelling response, and this relationship was impacted by environmental influences.
Conclusion: The use of a constant relationship of 80 % shelling biases grain yield prediction in multi-environment hybrid maize yield trials and thus reduces the rate of genetic gain in maize breeding programs.
Implications or significance: Taking into account the variations in the shelling percentage of the genotypes across sites in predicting grain yield from field weight improves the accuracy of genotype selection and the rate of genetic gain in maize breeding programs.

1. Introduction

Maize grain yield is a complex and quantitative meta-trait, controlled by many genes impacting many component traits. Effective yield gain in a plant breeding program therefore needs to target the improvement of multiple yield components simultaneously (Bello and Olaoye, 2009; Geetha and Jayaraman, 2000). Maize grain yield encompasses multiple component traits determined by parameters associated with the ear, the cob and the grain. Knowledge of the association between yield and its component traits and among the component traits themselves can increase the efficiency of selection in maize breeding programs (Bello and Olaoye, 2009; Raghu et al., 2011). Such

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understanding can inform which traits to target for selection, in addition to potential trade-offs that may occur between correlated component traits, and hence inform decisions on what strategies to use to improve yield without compromising other important traits. This last element is critical to take into account, because altering the expression of one trait is usually associated with a change in the expression of other traits if the two traits are correlated (Ahmad and Saleem, 2003).

The proportion of grain weight to ear weight is defined as the shelling percentage, and in maize, shelling percentage varies with genotypes, environments, and management (Horrocks and Zuber, 1970; Khan, 2015; Loesch et al., 1976; Sun et al., 2019). Loesch et al. (1976) reported that multiple factors had a significant effect on shelling percentage of hybrids, including the trial location, year, planting density and the interaction between these factors. Shelling percentage has been reported to be lower in multiple studies when under conditions with low soil nitrogen rather than optimal soil nitrogen (Amanullah et al., 2016; Jansen and Lübberstedt, 2012). The decrease observed in kernel size and kernel weight under such low soil nitrogen conditions contributes to the lower shelling percentage under such environments. Likewise, the same trend has been reported for soil phosphorus content in maize; where grain yield, grains per ear, thousand seed weight and shelling percentage were reported to increase when the rate of phosphorous fertilizer applied increased (Amanullah and Khan, 2015).

In maize, grain yield is positively correlated with ears per plant, ear weight, shelling percentage, plant height, and days to anthesis. However, there are contradicting reports from different studies. For example, Loesch et al., (1976) reported a negative association between cob length and grain yield. Similarly, Hallauer et al. (2010) reported that selection for long ears did not increase grain yield after multiple cycles of selections. As the influence of the environment and the interaction of environment with genotype (GEI) is important in quantitative (polygenic) traits, the knowledge and correct interpretation of GEI for target traits is crucial for genotype selection, as well as the relationship of these traits across environments.

Another critical consideration for improving efficiency of selection in maize breeding programs is the accuracy of phenotyping, which can be influenced by many factors including the trait measurement process. In almost all sub-Saharan African maize breeding programs, maize grain yield in breeding experimental plots is measured as the fresh weight of maize ears, which includes both the grain weight and cob weight. The weight of grain from each plot is estimated as a standard proportion of grain to total ear weight using a shelling percentage of 80 %. This approach assumes that there is no genetic, environment or GEI in shelling percentage which, if it were present, would likely result in reduced rates of genetic gain for grain yield if these data were used for selections.

Advanced statistical methods such as the linear mixed model (LMM) can contribute to improving the estimation of phenotypic values and are now widely used for the analysis of multi-environment (MET) data (Smith et al., 2005). The LMM can simultaneously incorporate models for spatial trend effects in individual field trials (Gilmour et al., 1997) and for genetic effects across multiple environments in the presence of GEI. A factor analytic variance structure (FALMM) (Smith et al., 2001) has been shown to improve the accuracy of prediction of the genetic value of a trait in METs for crop improvement programs (Kelly et al., 2007). The LMM framework is very flexible and has been extended for the analysis of multiple traits (De Faveri et al., 2015). Multi-trait models have been shown to increase the accuracy of prediction over single trait models (De Faveri et al., 2017, 2015, 2023; Smith et al., 2007). Multi-trait models improve the prediction accuracy of genetic values using the genetic correlation between traits or temporal correlations in multi-harvest data (De Faveri et al., 2015).

This study investigates the magnitude of genetic and environmental variation in shelling percentage and its impact on selection of maize genotypes in breeding trials from the Ethiopian mid-altitude maize breeding program. The relationship between CW, SW and FW is quantified in six hybrid maize multi-environment yield trials in three ways. The first analysis compares the grain yield estimated from FW using a standard shelling percentage of 80 % against the grain yield obtained from the measured GYS in tons per hectare and demonstrates the discrepancy of genotype selection in maize breeding yield trials using the shelling percentage adjustment of 80 %. The second analysis uses a bivariate linear mixed model to quantify the genetic variation for the component ear weight traits of CW and shelled grain weight across environments and the GEI variation in the relationship between these two traits, highlighting the risk of assuming a constant relationship between these traits. The third analysis uses a bivariate linear mixed model to estimate the average shelling percentage at each site as well as genetic deviations around this average shelling percentage, and proposes an efficient method for improved accuracy when predicting grain yield from the FW using a bivariate regression.

2. Materials and methods

2.1. Genetic material and field trials

The study was comprised of 478 maize hybrids adapted to the mid-altitude sub-humid maize growing agro-ecology of Ethiopia. This agro-ecology represents the major maize growing areas which account for more than 50 % of the maize production in the country. The hybrids were grown in six trials (sites) during the 2019 and 2020 main cropping seasons in Ethiopia, comprising two trials in 2019 and four trials in 2020. All trials were planted as a partially replicated designs laid out as a rectangular array of plots indexed by columns and rows, with 8 columns and varying number of rows (Table 1). The number of genotypes in each trial ranged from 237 to 250, and every trial had a subset of genotypes which were common across all trials. The minimum number of genotypes in common between each pair of trials was 40 (Table 2).

An experimental unit was a single row plot, 4.5 m long, spaced 0.75 m between rows and 0.25 m between plants. Two seeds were hand planted per hill and subsequently thinned to one plant per hill at 4 weeks after emergence, to give a final plant population density of 53,333 plants ha^{-1}. At all experimental sites, standard local agronomic practices including weeding and appropriate fertilizer applications were followed.

2.2. Trait measurements

Multiple traits were recorded on each experimental field plot. The ears were hand-harvested from each plot, then were weighed with the weights recorded as field weight (FW) in kg/plot. The ears were subsequently shelled with a maize sheller, and the shelled grain weight (SW) recorded in kg/plot. The moisture content (MOI) of the grain was determined from a subsample of the grain using a Dickey–John moisture meter. The cob weight (CW) in kg/plot was obtained by subtracting shelled weight from the FW. Grain yield in tons per hectare from the FW (GYF) was estimated based on a shelling percentage of 80 % and adjusted to a standard moisture content of 12.5 % as,

$$\mathrm{GYF} = \frac{\mathrm{FW}(100 - \mathrm{MOI})*10*0.8}{(100\text{-}12.5)*\mathrm{Plot\ size}}$$

where GYF is grain yield (t/ha), FW is field weight (kg/plot), MOI is the moisture content of the grain at harvesting time and plot size is in squared meters (m^2).

Grain yield in tons per hectare from the shelled grain weight (SW) was estimated by adjusting for a standard grain moisture content of 12.5 % as,

$$\mathrm{GYS} = \frac{\mathrm{SW}(100 - \mathrm{MOI})*10}{(100\text{-}12.5)*\mathrm{Plot\ size}}$$

where GYS is the grain yield in tons per hectare, SW is the shelled grain weight in kilogram per plot. For analysis purposes, the FW was also

Table 1
Number of columns, rows, genotypes in each trial, and altitude, latitude, and longitude of the sites where the trials are conducted.

Site	Management	Year	Column	Row	Genotype	Altitude (m asl)	Latitude (^{0}N)	Longitude (^{0}E)
19BKLN	Low N	2019	8	44	242	1610	9.09	37.04
19BKON	Optimum N	2019	8	44	259	1650	9.11	37.04
20BKO1	Optimum N	2020	8	56	240	1650	9.11	37.04
20BKO2	Optimum N	2020	8	58	250	1650	9.11	37.04
20MLO1	Optimum N	2020	8	56	240	1550	8.39	39.32
20MLO2	Optimum N	2020	8	58	249	1550	8.39	39.32

Table 2
Genotype concurrence between pair of sites. The diagonal shows the number of genotypes tested at each site and the off-diagonal shows genotypes in common between pairs of sites.

Site	19BKLN	19BKON	20BKO1	20BKO2	20MLO1	20MLO2
19BKLN	242					
19BKON	242	259				
20BKO1	173	180	240			
20BKO2	40	40	91	250		
20MLO1	173	180	240	91	240	
20MLO2	40	40	91	249	91	249

converted to tons/hectare.

2.3. Statistical methods

Two separate univariate multi-environment models accounting for GEI effects were fitted in a LMM framework to GYS (hereafter MET-1) and GYF (hereafter MET-2), respectively. The aim of this analysis was to demonstrate the difference in yield predictions from the shelled grain weight and grain yield estimated using 80 % shelling. Following this analysis, two separate bivariate models were fitted in a LMM framework. The first bivariate model was for SW and CW (hereafter bivMET-1) with the aim of demonstrating the genetic relationship for these component traits of yield across sites. The second bivariate model explored the relationship of the two traits of FW and SW across sites (here after bivMET-2). This analysis was used to estimate the actual shelling percentage at each site, and contrast this with the assumed fixed shelling percentage of 80 %. The statistical procedures to model the univariate and bivariate models are outlined in the following sections.

2.3.1. Modelling univariate multi-environment trials for genotype by environment effects

The baseline LMM for the MET analysis of a single trait is presented as follows. Consider a series of field experiments for a total of n plots, where $n=\sum_{i=1}^{p} n_i$ and n_i is the number of plots in trial i, where $i=1,\ldots,p$ for p trials. Each trial is laid out in a rectangular array of r_i rows and c_i columns ($n_i = r_i\, c_i$). The series of field trials contains a total of m genotypes or hybrids, noting that not all genotypes need to occur in each trial. The linear mixed model for a response variable $\boldsymbol{y}$ is

$$\boldsymbol{y}=\boldsymbol{X\tau}+\boldsymbol{Z}_g\boldsymbol{u}_g+\boldsymbol{Z}_o\boldsymbol{u}_o+\boldsymbol{\varepsilon} \tag{1}$$

where $\boldsymbol{\tau}^{(t\times1)}$ is a vector of fixed effects with associated design matrix $\boldsymbol{X}^{(n\times t)}$ of full column rank, $\boldsymbol{u}_g^{(pm\times1)}$ contains the random genotype by environment effects with indicator matrix $\boldsymbol{Z}_g^{(n\times pm)}$, $\boldsymbol{u}_o^{(b\times1)}$ is the vector of non-genetic random effects with associated design matrix $\boldsymbol{Z}_o^{(n\times b)}$ and $\boldsymbol{\varepsilon}^{(n\times1)}$ is the vector of residual errors across all trials. The vector $\boldsymbol{\tau}$ contains the grand mean for each trial (p fixed effects) and may also include fixed effects due to global trend (Gilmour et al. 1997). The vector $\boldsymbol{u}_o$ includes effects associated with the experimental design of the trial as well as terms for modeling extraneous variation which arise from experimental procedures (Gilmour et al. 1997). It is assumed that the joint distribution of ($\boldsymbol{u}_g$, $\boldsymbol{u}_o$, $\boldsymbol{\varepsilon}$) is multivariate normal with zero mean.

The variance of the random residual effects, $\boldsymbol{\varepsilon}$ can be expressed as the direct sum of p separable variance structures arising from n_i plots in each trial, independently for p trials.

$$\mathbf{R}=\oplus_{j=1}^{p}\quad \boldsymbol{R}_j=\begin{bmatrix}\boldsymbol{R}_1 & 0 & \ldots & 0\\ 0 & \boldsymbol{R}_2 & \ldots & 0\\ \vdots & \vdots & \ddots & \vdots\\ 0 & 0 & 0 & \boldsymbol{R}_p\end{bmatrix}$$

where $\boldsymbol{R}_j$is an $n_j\times n_j$ variance matrix between plots. In its simplest form $\boldsymbol{R}_j=\sigma^2_{\varepsilon_j}\boldsymbol{I}_{n_j}$. Following the spatial model for residual errors using a separable variance structure for correlation in the column and row directions, respectively, $\boldsymbol{R}_j=\sigma^2_{\varepsilon_j}\boldsymbol{\Sigma}_{c_j}\otimes\ \boldsymbol{\Sigma}_{r_j}$, where $\boldsymbol{\Sigma}_{c_j}$is the first order autoregressive correlation matrix for and $\boldsymbol{\Sigma}_{c_j}$ is similarly for columns (Gilmour et al., 1997).

The variance matrix $\boldsymbol{G}_g$ is a separable variance structure arising from a compound term for $\boldsymbol{u}_g$ formed from m genotypes by p environments. The separable structure assumes that

$$\boldsymbol{G}_g \quad = \quad \boldsymbol{G}_e \quad \otimes\boldsymbol{I}_m$$

where $\boldsymbol{G}_e$ is the $p\times p$ variance matrix between environments, also commonly known as the genetic variance matrix between environments, and $\boldsymbol{I}_m$ is an identity matrix of order m. A factor analytic form was fitted to the variance matrix $\boldsymbol{G}_e$ (Smith et al. 2001).

2.3.2. Modelling bivariate multi-environment trials for genotype by environment effects

The linear mixed model across multiple environments can be extended for two traits as follows.

$$\boldsymbol{y}^*=(\boldsymbol{I}_2\otimes\boldsymbol{X})\boldsymbol{\tau}_t+(\boldsymbol{I}_2\otimes\boldsymbol{Z}_g)\boldsymbol{u}_{tg}+(\boldsymbol{I}_2\otimes\boldsymbol{Z}_o)\boldsymbol{u}_{to}+\boldsymbol{\varepsilon}^* \tag{2}$$

where $\boldsymbol{y}$* is a $2n\times1$ vector of data for two traits measured on n plots, $n=\sum_{i=1}^{p} n_i$ and n_i is the number of plots in trial i, where $i=1,\ldots,p$ for p trials. The fixed effect vector, $\boldsymbol{\tau}_t$ with associated design matrix $\boldsymbol{X}$, is typically a $2p\times1$ vector of trial-trait effects. The vector $\boldsymbol{u}_{tg}$ is a $2pm\times1$ vector of random genotype effects for each trial-trait combination, with corresponding design matrix $\boldsymbol{Z}_g$. The vector of non-genetic random effects, $\boldsymbol{u}_{to}$ with associated design matrix $\boldsymbol{Z}_o$ consists of sub-vectors corresponding to the design factors of each trial for each trait, and $\boldsymbol{\varepsilon}$* is the vector of residual errors across all trial-trait combinations. As previously, the vector $\boldsymbol{\tau}_t$ may also include fixed effects due to global trend in each trial (Gilmour et al. 1997) and the vector $\boldsymbol{u}_{to}$ may also include terms for modeling extraneous variation which arises from spatial trend in each trial (Gilmour et al. 1997).

The variance of the random effects, $\boldsymbol{u}_{tg}$ can be expressed as a separable variance structure arising from a compound term for $\boldsymbol{u}_{tg}$ formed from m genotypes for two traits at p environments. The separable structure assumes that

$$\boldsymbol{G}_g \quad = \quad \boldsymbol{G}_{tt} \quad \otimes \quad \boldsymbol{I}_m$$

where $\boldsymbol{G}_{tt}$ is the $2p\times2p$ variance matrix between trial-trait combinations.

The variance of the random residual effects, $\boldsymbol{\varepsilon}$*, was modelled using the two-dimensional invariant multivariate AR1 (2DIMVAR1) model of

De Faveri et al. (2017), (2023) as

$\boldsymbol{R}_j = \sum_{i=1}^{2} \boldsymbol{\Sigma}_{cs_j} \otimes \boldsymbol{\Sigma}_{rs_j} \otimes \boldsymbol{p}_{s_j}\boldsymbol{p}_{s_j}^T$, where, $\boldsymbol{\Sigma}_{cs_j}$ and $\boldsymbol{\Sigma}_{rs_j}$ are the correlation matrices for first-order autoregressive processes for rows and columns of site *j*, and $\boldsymbol{p}$ is a canonical transformation matrix that diagonalizes the two matrices. This was reverted to the separable residual variance structure (Ganesalingam et al. 2013) when it was not a significant improvement in model fit.

The Akaike information criterion (AIC) (Akaike,1998) and log-likelihood ratio test were used to determine the order of the most parsimonious FA model within each data set, where all comparisons were made between nested models. All the analyses were undertaken in the R environment (R Core Team 2023, version 4.0.5), using the ASReml-R (Butler et al., 2017) package, version 4.1.

2.3.3. *Estimation of shelling percentage from the bivariate model*

The shelling percentage of genotypes at each site was estimated from the bivMET-2 (Eq. 2) as follows. Consider the partitioning of the random genotype effects, $\boldsymbol{u}_{tg}$for the trait combinations in trial *j*,

$$\boldsymbol{u}_{\mathrm{tg}_j} = \begin{bmatrix} \mathbf{u}_{tg_{1j}} \\ \mathbf{u}_{tg_{2j}} \end{bmatrix} \sim N\left(\begin{matrix} \mathbf{0} \\ \mathbf{0} \end{matrix}, \begin{bmatrix} \sigma^2_{g_{1j}} & \\ \sigma_{tg_{12j}} & \sigma^2_{g_{2j}} \end{bmatrix} \otimes \mathbf{I}_m \right)$$

where $\sigma^2_{g_{1j}}$ is the variance of genotype effects for FW, $\sigma^2_{g_{2j}}$ is the variance of genotype effects for GYS and $\sigma_{tg_{12j}}$ is the covariance between FW and GYS in trial *j*. A measure of shelling percentage deviation, $\widetilde{\boldsymbol{u}}_s$, is formed from the regression of the estimated genotype effects for fresh weight of ear, against the estimated genotype effects for GYS as

$$\widetilde{\boldsymbol{u}}_{\mathrm{tg}_{2j}} = \widehat{\beta}\ \widetilde{\boldsymbol{u}}_{\mathrm{tg}_{1j}} + \widetilde{\boldsymbol{u}}_s, \tag{3}$$

where $\widehat{\beta} = \frac{\sigma_{tg_{12j}}}{\sigma^2_{g_{1j}}}$ is the average shelling percentage for that environment.

Then, $\mathrm{var}\left(\begin{bmatrix} \widetilde{\boldsymbol{u}}_{g_{1j}} \\ \widetilde{\boldsymbol{u}}_s \end{bmatrix}\right) = \begin{bmatrix} \sigma^2_{tg_{1j}} & 0 \\ 0 & \sigma^2_s \end{bmatrix} \otimes \mathbf{I}_m$, where $\sigma^2_s = \sigma^2_{tg_{2j}}(1 - \rho^2)$, and $\rho^2 = \frac{\left(\sigma_{tg_{12j}}\right)^2}{\sigma^2_{tg_{1j}}\sigma^2_{tg_{2j}}}$, and all σ_i^2 and σ_i terms are Residual maximum likelihood (REML) estimates of variance parameters.

From the above equation, the average shelling percentage at each site was estimated from the FW and SW bivariate model as the slope of the regression line. $\widehat{\beta}$. The deviation from the regression line, $\widetilde{\boldsymbol{u}}_s$, was the genetic deviation from the average shelling percentage for each genotype at each site.

3. Results

The following four statistical models were fitted to the data sets; firstly, two separate univariate models were fitted to GYS and GYF, respectively, and secondly, two separate bivariate models were fitted in a LMM framework. The first bivariate model was fitted to CW and SW to partition the genetic relationship of these traits. The second bivariate model was fitted to FW and SW to estimate the average shelling percentage of the genotypes at each site.

3.1. *The multi-environment trial analysis of shelled grain yield and grain yield from field weight*

In the univariate MET analysis (Eq. 1) for GYS and GYF, an FA2 model was fitted for GYS and an FA1 model was fitted for GYF, based on model comparison for increasing order of FA using the AIC.

Genetic correlations between each pair of environments for GYS ranged from 0.19 (20BKO2 and 19BKLN) to 0.91 (20BKO1 and 20BKO2) indicating a range from strong to weak GEI between environments. Our experience suggests that a genetic correlation value of 0.6 between environments is a threshold above which there is no change in the ranking of genotypes between two sites. The environment 19BKLN exhibited the highest GEI where all genetic correlations with the other environments were lower than 0.6. Similarly, the genetic correlation between each pair of environments for GYF ranged from 0.26 to 0.87 indicating the presence of strong to weak GEI. As observed in GYS, the 19BKLN environment had genetic correlations less than 0.6 with the remaining environments indicating the presence of strong GEI (data not shown).

Ranking of the top 20 % of the genotypes from GYS and GYF showed differences across sites (Fig. 1). The presence of rank change in the top 20 % of genotypes between the two traits indicated large discrepancies in selections made depending on the estimated versus measured trait. This means that one can select different genotypes using the constant relationship of 80 % shelling from the selections for GYS, yet aiming at improving the same trait, grain yield. The number of matched and mismatched genotypes in the top 20 % of the genotypes based on the performance of GYS and GYF varies across sites indicting that the degree of discrepancy in estimating grain yield using 80 % shelling is site dependent. It was also clear that the degree of rank discrepancy was more intense at the stressed sites, 19BKLN. Considering the list of top 20 % of the genotypes at each site, the percentage of mismatched genotypes was 34.4 % at 19BKLN, 33 % at 19BKON, 27 % at 20BKO1, 27 % at 20BKO2, 31.6 % at 20MLO1 and 27 % at 20MLO2.

3.2. *A bivariate multi-environment trial analysis of cob weight and shelled grain weight (bivMET-1)*

The bivMET-1 model was based on a factor analytic model of order 4 (FA4) for the trial-trait by genotype effects (Eq. 2). For the residual correlation between traits, the 2DIMVAR1(De Faveri et al., 2017; De Faveri et al., 2023) structure between traits was significant at two locations (19BKLN and 20MLO2). A separable residual variance model (Ganesalingam et al., 2013) was fitted for the correlation between traits at the remaining four locations. This final model was selected from a range of orders of FA models and different trait residual correlation models using the AIC for model comparison.

A summary of the results from the bivMET-1 model for CW and SW is presented in Table 3. Genetic variance for CW across sites ranged from 0.029 at 19BKLN to 0.245 at 19BKON. The error variance for CW ranged from 0.059 at 20BKO2 to 0.349 at 20MLO2. Likewise, there was substantial variation for SW across sites with genetic variance ranging from 0.199 at 19BKLN to 2.822 at 20BKO2. The residual error variance for SW varied from 1.057 at 20BKO2 to 3.003 at 20MLO2. The genetic relationship between the two traits (CW and SW) at the same site varied from 0.39 at 19BKLN to 0.71 at 19BKON. The residual trait correlation between CW and SW also ranged from 0.63 at 20MLO1 to 0.79 at 19BKLN (Table 3).

The genetic correlation between sites for SW, CW and genetic correlation between CW and SW across environments is shown in Fig. 2. The genetic correlation between sites for SW ranged from 0.19 (between 19BKLN & 20BKO2) to 0.86 (between 20BKO1 & 20BKO2). Site 19BKLN, exhibited lower genetic correlations (less than 0.4) except with sites 19BKON and 20MLO2 indicating the presence of GEI for SW (Fig. 2, upper right).

Similarly, the genetic correlation between pair of sites for CW ranged from 0.38 (19BKLN & 20MLO1) to 0.86 (20MLO1 & 20MLO2). This indicates the presence of GEI for CW (Fig. 2, lower left). The genetic correlations between sites for SW and CW revealed that the genetic predictions for both traits varied with environment.

The genetic correlations between traits at the same environment (Fig. 2, diagonal values of the lower right) and correlations between traits at different environments (Fig. 2, off-diagonal values of the lower right) from the bivariate analysis of CW and SW revealed that there is

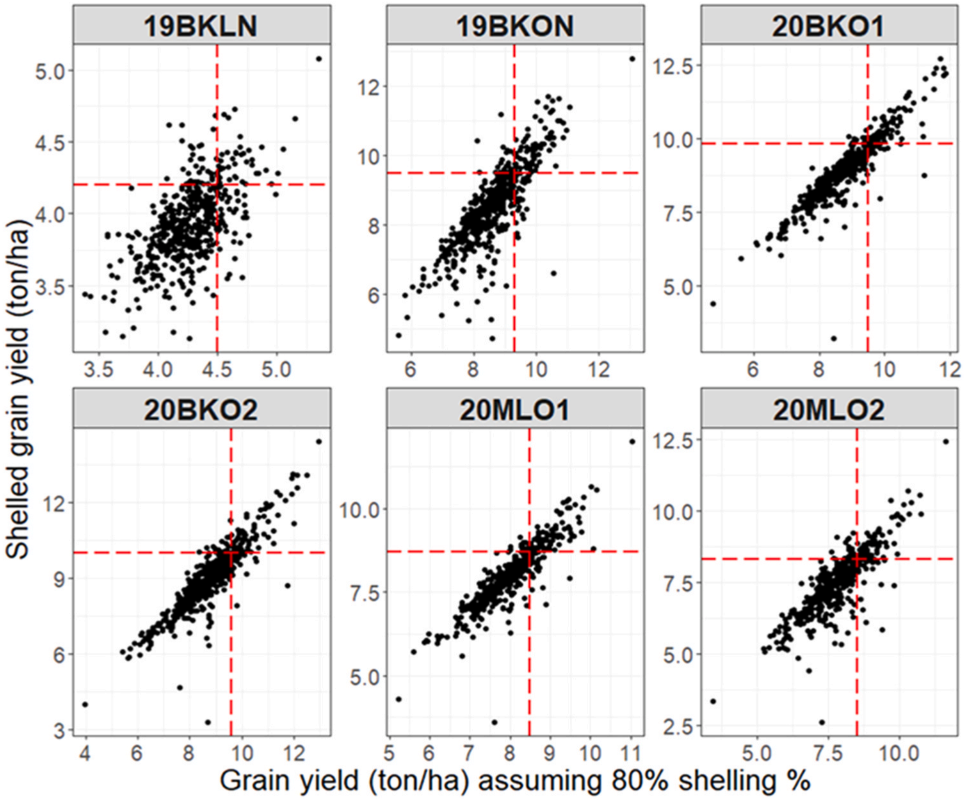


Fig. 1. Grain yield predictions of shelled grain yield (GYS) against GYF from the univariate MET models. The dotted red line shows the top 20 % of the selections, with the vertical line defining selection cut-off for genotypes using the estimated grain weight assuming 80 % shelling, and the horizontal line defining the selection cut-off for measured grain yield (GYS).

Table 3
Genetic variances for sites, genetic correlations between SW and CW at each site, residual correlations from bivMET-1 model.

	σ_g^2		Genetic trait correlation	σ_ε^2		Residual trait correlation
Sites	CW	SW		CW	SW	
19BKLN	0.029	0.199	0.39	0.157	1.102	0.79
19BKON	0.245	2.975	0.71	0.168	1.766	0.69
20BKO1	0.166	2.000	0.50	0.082	1.101	0.72
20BKO2	0.173	2.822	0.61	0.059	1.057	0.75
20MLO1	0.111	1.358	0.47	0.112	1.349	0.63
20MLO2	0.192	1.964	0.69	0.349	3.003	0.76

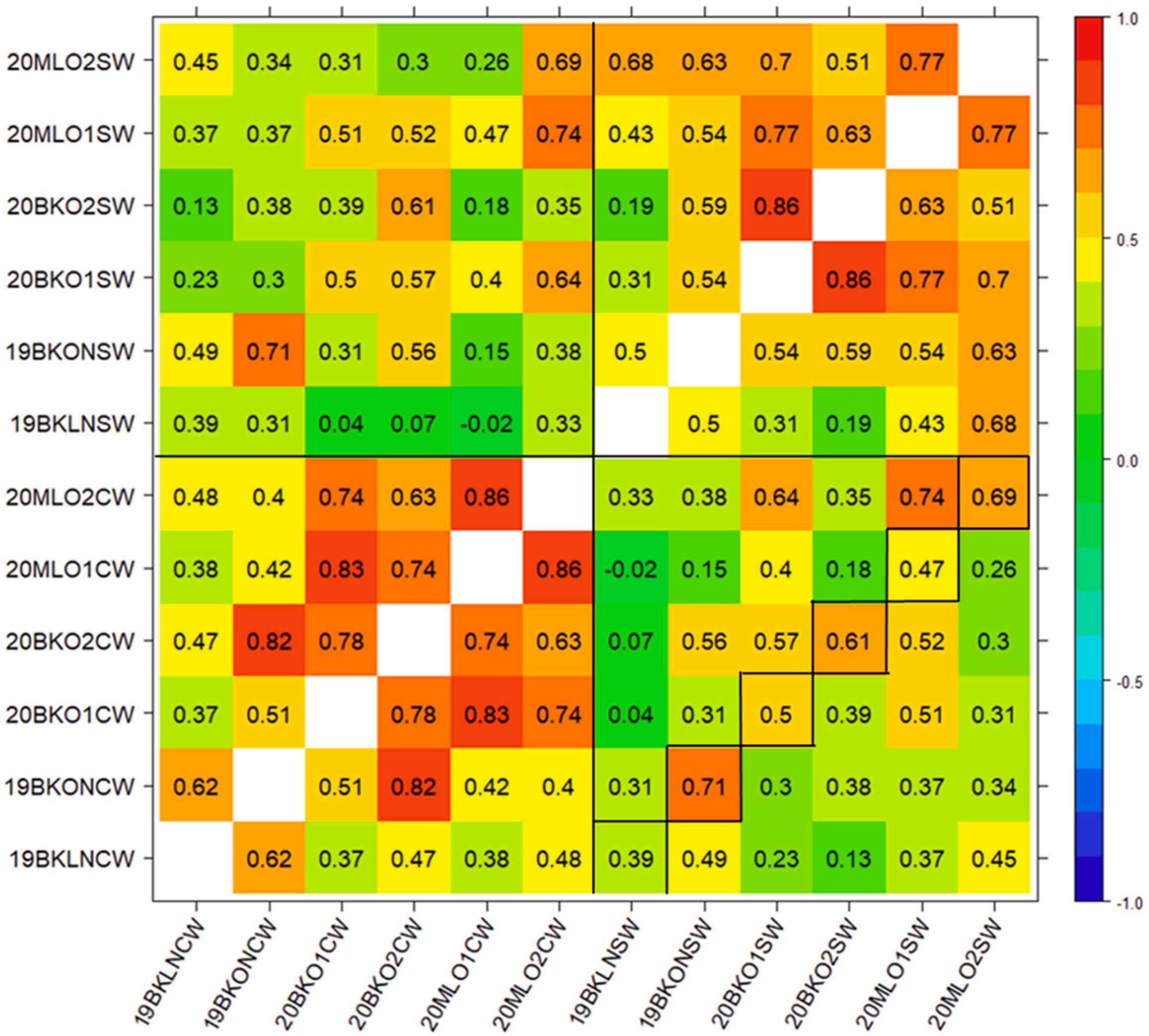
extensive variation in relationship of the two traits across sites. The genetic correlations between the two traits varied across environments with values 0.39 at 19BKLN, 0.71 at 19BKON, 0.50 at 20BKO1, 0.61 at 20BKO2, 0.47 at 20MLO1, and 0.69 at 20MLO2. This indicated that the relationship of the two traits varied across environments and the use of a constant relationship between these traits leads to inaccurate adjustments assuming a standard relationship for all genotypes across all environments. It is also important to note that the relationship of the CW and SW is weaker in stressed environments than the optimum environments.

Furthermore, there was a specific relationship between CW and SW at each different pair of environments (Fig. 2, off-diagonal values of the lower right). For example, the genetic correlation between CW at 20MLO1 and SW at 19BKLN was −0.02, while the genetic correlation between CW at 19BKLN & SW at 20MLO1 was 0.37. This indicates the unique relationship of the two traits at individual sites. The differing correlations indicate that the highest CW at one site does not reflect the higher value of grain weight (SW) at the same site, and vice versa. The exception is for CW at 20MLO2 with SW at 20BKO1 & 20MLO1, where the correlations were moderately high, otherwise the correlations between the remaining sites were relatively low indicating the presence of weak relationship between the traits across sites. This indicated that the relationship of these two traits is highly influenced by environmental factors.

3.3. *A bivariate multi-environment trial analysis of field weight and shelled weight (bivMET-2)*

The second bivariate model (bivMET-2) was fitted to estimate the average shelling percentage at each environment and to estimate the shelling percentage deviation for each genotype at each environment. An FA model of order 5 was the chosen model for the genetic variance matrix across trial-trait combinations. A separable residual variance model with heterogeneous genetic correlation structure between the traits (Ganesalingam et al., 2013) provided the best model fit for four sites *viz* 19BKON, 20BKO1, 20BKO2 and 20MLO1. For the remaining two sites, 19BKLN and 20MLO2, a heterogenous genetic variance structure between traits with the 2DIMVAR1 residual variance model (De Faveri et al., 2017, De Faveri et al., 2023) was shown to have significant improvement in model fit.

Results of variance components, genetic and residual trait correlations, and non-genetic correlations from the bivariate model fitted to FW and SW are presented in Table 4. There were genetic variations for both FW and SW across environments. The genetic variance for FW ranged from 0.364 at 19BKLN to 3.814 at 19BKON. The genetic variance for SW also varied across sites ranging from 0.229 at 19BKLN to 2.826 at 20BKO2. The genetic variances for FW were slightly greater than the genetic variances of SW in magnitude at all sites. The genetic correlations between FW and SW across sites was quite high, ranging from 0.97 to 0.99. The residual error correlation between the traits was also high ranging from 0.92 to 0.98.



	19BKLNCW	19BKONCW	20BKO1CW	20BKO2CW	20MLO1CW	20MLO2CW	19BKLNSW	19BKONSW	20BKO1SW	20BKO2SW	20MLO1SW	20MLO2SW
20MLO2SW	0.45	0.34	0.31	0.3	0.26	0.69	0.68	0.63	0.7	0.51	0.77	
20MLO1SW	0.37	0.37	0.51	0.52	0.47	0.74	0.43	0.54	0.77	0.63		0.77
20BKO2SW	0.13	0.38	0.39	0.61	0.18	0.35	0.19	0.59	0.86		0.63	0.51
20BKO1SW	0.23	0.3	0.5	0.57	0.4	0.64	0.31	0.54		0.86	0.77	0.7
19BKONSW	0.49	0.71	0.31	0.56	0.15	0.38	0.5		0.54	0.59	0.54	0.63
19BKLNSW	0.39	0.31	0.04	0.07	-0.02	0.33		0.5	0.31	0.19	0.43	0.68
20MLO2CW	0.48	0.4	0.74	0.63	0.86		0.33	0.38	0.64	0.35	0.74	0.69
20MLO1CW	0.38	0.42	0.83	0.74		0.86	-0.02	0.15	0.4	0.18	0.47	0.26
20BKO2CW	0.47	0.82	0.78		0.74	0.63	0.07	0.56	0.57	0.61	0.52	0.3
20BKO1CW	0.37	0.51		0.78	0.83	0.74	0.04	0.31	0.5	0.39	0.51	0.31
19BKONCW	0.62		0.51	0.82	0.42	0.4	0.31	0.71	0.3	0.38	0.37	0.34
19BKLNCW		0.62	0.37	0.47	0.38	0.48	0.39	0.49	0.23	0.13	0.37	0.45

Fig. 2. Genetic correlations between sites for shelled grain weight (SW, upper right) and cob weight (CW, lower left) and the genetic correlations between SW and CW (upper left or lower right) across sites from bivMET-1. The diagonal values are the genetic correlations between SW and CW at the same site and the off-diagonal values are the genetic correlations between SW and CW at different pairs of sites (lower right).

Table 4

Genetic variances, error variances, genetic correlation between field weight (FW) and shelled weight (SW), residual trait correlations, slope of the regression and residual autocorrelations along the rows and columns from bivMET-2 model.

Site	σ_g^2		σ_ε^2		Genetic Trait correlation	Residual Trait correlation	$\widehat{\beta}$
	FW	SW	FW	SW			
19BKLN	0.364	0.229	2.142	1.266	0.97	0.98	0.77
19BKON	3.814	2.506	3.362	1.897	0.97	0.92	0.79
20BKO1	2.674	1.904	1.578	1.054	0.97	0.98	0.82
20BKO2	3.704	2.826	1.512	1.079	0.98	0.98	0.86
20MLO1	1.871	1.309	1.986	1.428	0.97	0.96	0.81
20MLO2	3.213	2.067	4.909	2.989	0.99	0.98	0.79

The genetic correlations between sites for FW and SW and the covariance of both FW and SW across sites from the bivariate MET model (bivMET-2) are presented in the supplementary material. The genetic correlation between pairs of sites for FW varied from 0.30 (20MLO1 and 19BKLN) to 0.89 (20BKO2 and 20BKO1) indicating the presence of GEI for this trait. Similarly, the genetic correlation between pairs of sites for SW also ranged from 0.34 (20MLO1 and 19BKLN) to 0.91 (20BKO2 and 20BKO1). There was a strong genetic trait correlation between FW and SW across sites. Nevertheless, the correlation between the two traits between pairs of sites varied across sites from weak to strong association.

The average shelling percentage at each site was estimated as the slope of the regression line using the bivariate regression (Eq. 2, from bivMET-2 model). The shelling percentage of each genotype was estimated as the deviation from the regression line at each site (Eq. 3). The shelling percentage of genotypes at each site ranged from 67.0 to 82.1 at 19BKLN, 64.8–85.8 at 19BKON, 61.8–91.3 at 20BKO1, 54.7–88.5 at 20BKO2, 69.4–86.1 at 20MLO1 and 67.6–85.7 at 20MLO2. The average shelling percentage of the genotypes at each of the sites was 76.4 at 19BKLN, 80.9 at 19BKON, 81.7 at 20BKO1, 82.3 at 20BKO2, 80.5 at

20MLO1 and 78.5 at 20MLO2.

4. Discussion

In maize breeding programs, thousands of combinations of parent lines are evaluated every season to identify potential hybrids with high yield and good agronomic characteristics to be promoted to later stages of variety evaluation or to be released. The performance of these experimental hybrids needs to be evaluated in field experiments in different environments over several seasons. However, the resources available for breeding programs are always limited, and field evaluation of hybrids is costly and labor-intensive, making phenotyping a major bottleneck for crop improvement (Crossa et al., 2017). In maize breeding programs in Ethiopia and elsewhere in Africa, where mechanization is limited, it is very challenging and costly to manually thresh (shell) each plot in every site in the MET. For this reason, most maize breeding programs in SSA estimate maize grain yield using the correlated trait of field weight as a surrogate for grain yield. The common practice is to assume a constant ratio of 0.8 between grain weight to cob weight (i.e shelling percentage of 80 %) for every genotype at every site (Abakemal et al., 2016; Ertiro et al., 2013; Otim et al., 2022; Rezende et al., 2020). This approach assumes that there is no genetic, environmental or GEI in shelling percentage in contrary to the information in the literature (Horrocks and Zuber, 1970; Loesch et al., 1976; Sun et al., 2019). The variation of shelling percentage across sites and genotypes would contribute to slower rates of genetic gain, when assumed as a constant for every genotype across sites for estimating grain yield. In this study, we demonstrated that there is genotype selection discrepancy in hybrid maize METs by using this fixed relationship of grain to cob ratio (shelling percentage of 80 %) as opposed to the measured grain yield. We further demonstrated that the relationship between CW and SW varies among genotypes and across sites, indicating the assumption of fixed shelling percentage for every genotype at every site potentially biases genotype selection in hybrid maize METs and thus slows the rate of genetic gain in breeding programs.

4.1. A measure of shelled grain weight highlights a problem with the assumption of shelling percentage

The difference in ranking of the genotypes between GYS and GYF biases genotype selection and this has a negative impact on the rate of genetic progress in breeding programs. Breeding programs often optimize their phenotyping operations with the aim of improving their efficiency and ultimately the rate of genetic gain. However, care must be taken that these optimization processes do not compromise the accuracy of genotype selection and the genetic gain that could be achieved in the long term. To enhance the efficiency of breeding programs, advanced statistical models have been used to support both predictive and analytic steps of genotype selection in breeding programs (Cooper et al., 2014; Crossa et al., 2021). Measuring SW in experimental plots in maize breeding trials results in extra cost, both in terms of money and time. However, given the level of genotype selection discrepancy observed in this study, the extra cost investment to shell a subset of genotypes at each site to estimate shelled weight of all genotypes from the FW far outweighs short-term cost savings in phenotyping operations and additionally contributes to increasing long-term genetic gain.

4.2. Both cob weight and shelled weight and their relationship vary across sites

The first bivariate model (bivMET-1) demonstrates that there is genetic, environmental and GEI for both CW and SW, thus highlighting the risks associated with the current practice of assuming a fixed shelling percentage for every genotype at every site. These findings support existing reports in the literature, where grain to cob ratio varies across genotypes, environment and management (Horrocks and Zuber, 1970; Khan, 2015; Loesch et al., 1976; Sun et al., 2019). Furthermore, the relationship between these two traits and how it varied across sites is a critical consideration for selection in a breeding program. The effect of GEI on the relationship of SW and CW is profoundly strong. The genetic correlations of the two traits (CW and SW, the diagonal values the lower right of Fig. 2) substantially varied across sites from weak positive to moderately large indicating the deviation from the assumption of a constant relationship between these two traits across sites. Thus, the use of a constant relationship of these traits across environments is not accurate. Most importantly, the relationship between the traits at a given pair of sites is not symmetrical. A pair of sites has different correlation values depending on which of the two traits was measured at which site, showing the significance of the influence of GEI on these traits (off diagonal values of the lower right of Fig. 2).

4.3. The use of fixed shelling percentage in estimating grain yield is inaccurate

The second bivariate model (bivMET-2) allowed us to estimate the average shelling percentage for each site, and the shelling percentage deviation for each genotype within each site. In this study, the genetic variations observed for both FW and SW showed marked deviations from the assumption of using 80 % shelling percentage across sites for all genotypes. While there was a relatively high positive genetic correlation between these traits (Table 4), the average shelling percentage of genotypes varied across environments, with stressed sites having lower shelling percentage values than high-yielding sites (Fig. 3). This shows that under environmental stress, grain size and weight is impacted by the amount of nitrogen, and this directly affects the shelling percentage (Jansen and Lübberstedt, 2012; Varvel and Wilhelm, 2008). Importantly, there were also genetic deviations from this average shelling percentage at each site, and these genetic deviations interacted with environments. The sophisticated statistical analysis model allowed us to estimate these deviations independently of the field weight, and these deviations quantify the genetic bias in assuming a constant shelling percentage. For the breeding program, ignoring these variations in shelling percentage in the genotypes and sites will incur costs in the long-term to realize genetic gain.

The strong genetic correlation between FW and SW across environments (Table 4) indicates that measuring one can reliably serve as a proxy for the other, an existing method for assessing yield based on FW. However, it is crucial to recognize that high correlations between traits may not fully capture the complexity of their shared genetic architecture. This issue is illustrated by the observed prediction discrepancies between GYF and GYS. Therefore, breeders should not solely rely on these high genetic correlations. Instead, they should carefully consider the target trait of interest and how genetic correlations are applied to ensure accurate measurements of surrogate traits. Developing predictive models that can concurrently account for the genetic variations in both traits (FW & SW) may offer a more reliable approach.

5. Conclusion

This study set out to examine the relationship between SW and CW in hybrid maize METs. The results of this investigation show that the two traits and their relationship varied across sites indicating that the use of a fixed shelling percentage for estimating grain yield in hybrid maize METs biases genotype selection and consequently slows the rate of genetic gain in breeding programs. Given the variations observed for CW, SW and the shelling percentage across sites, relying on a fixed shelling percentage for every genotype at every site leads to inaccurate genotype selections and a low rate of genetic gain in breeding programs. Further work is being undertaken to develop a predictive model for GYS from a sub-sample of genotypes shelled at each site, taking into account the variation of both components of shelling percentage across different sites. This will increase the accuracy of genotype predictions and

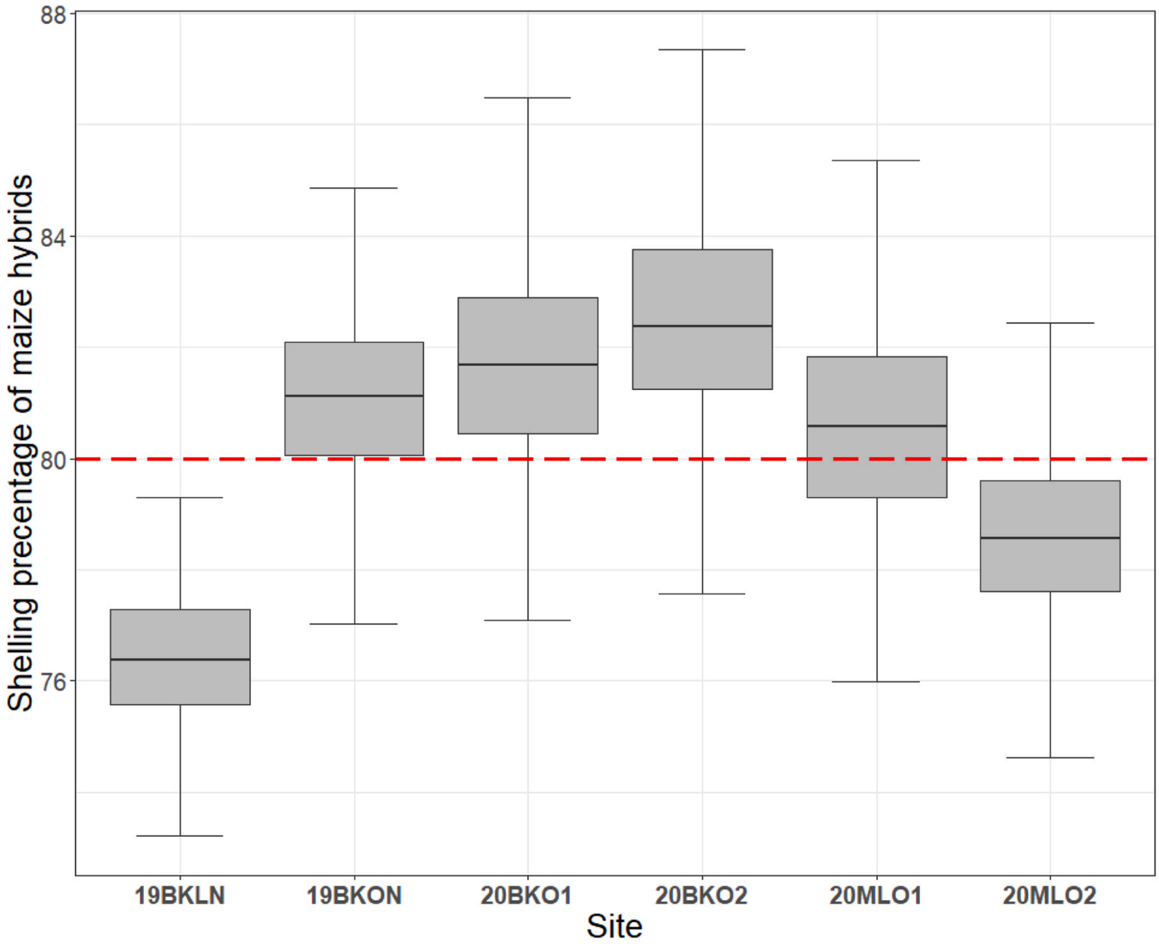


Fig. 3. Estimated shelling percentage of genotypes at each site from the bivariate model (bivMET-2). The dotted horizontal line shows 80 % shelling, the value used for the current practice to calculate grain yield from harvested field weight in breeding trials in Ethiopia and elsewhere in SSA.

improve the efficiency of grain yield phenotyping in maize METs ensuring the realization of improved long-term genetic gain in maize breeding programs.

CRediT authorship contribution statement

Tolera Keno: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Emma Mace:** Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing. **Alison Kelly:** Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing – review & editing. **Ian Godwin:** Investigation, Writing – review & editing. **David Jordan:** Conceptualization, Project administration, Supervision, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data availability

Data will be made available on request.

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Declarations

The authors declare that they have no relevant financial or non-financial interests to disclose.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2024.109437.

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