



Phenotypic variability, heritability and trait associations among eighteen maize (*Zea mays* L.) accessions under spring conditions in Faisalabad, Pakistan.

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Abstract

Genetic variation in agronomic traits is important for selecting maize accessions for breeding. 18 maize accessions obtained from the Maize Breeding and Genetics Laboratory, University of Agriculture, Faisalabad, Pakistan, were grown in a randomized complete block design with three replications during spring 2023. Ten morphological and grain traits were recorded: plant height, ear height, internode diameter, internode length, cob length, rows per cob, seeds per row, cob diameter, pith diameter, and 100-grain weight. Analysis of variance showed significant differences among accessions for all ten traits, although internode diameter was only marginally significant ($P = 0.049$). Broad-sense heritability was high for plant height (95%) and cob diameter (68%), moderate for most other traits (34 to 51%), and low for internode diameter (24%). Genetic advance as a percentage of the mean was highest for pith diameter (27.3%) and plant height (24.5%). Accessions 2068, 43, 314 and 113 had the heaviest grains (36.2 to 40.1 g per 100 grains), and the first three accessions 2068, 43, and 314 were also shorter than the group means (138 to 153 cm against 156 cm). Plant height was negatively correlated with 100-grain weight ($r = -0.33$), while seeds per row ($r = 0.30$) and ear height ($r = 0.29$) were positively correlated with it. The first four principal components of the accession mean explained 80.3% of the variation, and Ward clustering separated the accessions into four groups. The results point to accessions worth testing as parents. These results identify accessions 2068, 43, 314, and 113 as promising candidates for further breeding evaluation because of their high 100-grain weight.

Keywords: maize, variability, heritability, genetic advance, correlation, principal component analysis.

Introduction

Maize (*Zea mays* L.) is grown across the world as food, livestock feed, and industrial raw material (Erenstein et al., 2022). In Pakistan, it contributes about 3.2% of agricultural value added and 0.7% of gross domestic product (Ministry of Finance, Government of Pakistan, 2022). Improving maize productivity requires the identification and effective use of genetic variation in agronomically important traits. Evaluating genetic variability and associations among agronomic traits can help identify promising maize genotypes for further breeding and crop improvement (Bartaula et al., 2019).

A variety can be improved only as far as the available genetic variation allows (Govindaraj et al., 2015). Breeders often work with a small set of closely related germplasm, which narrows the base for selection (Prasanna, 2012). Before parents are chosen, the accessions on hand need to be described: how much they differ, how much of that difference is passed on to progeny, and how the traits relate to one another (Govindaraj et al., 2015; Tadesse et al., 2018a).

Variability studies in maize commonly estimate the

genotypic and phenotypic coefficients of variation, broad-sense heritability, and genetic advance (Alam et al., 2022; Shrestha et al., 2023). Heritability read alone can mislead, so it is interpreted together with genetic advance (Robinson & Comstock, 1955). Correlation analysis then shows which traits move with grain weight and yield, and multivariate methods such as principal component analysis and cluster analysis summarize how the accessions group (Mohammadi & Prasanna, n.d.; Mubai et al., 2020).

Therefore, this study aimed to evaluate genetic variation among 18 maize accessions for ten morphological and grain traits and to identify accessions with desirable trait characteristics for further breeding evaluation. It also aimed to examine associations among the measured traits, particularly their relationships with 100-grain weight

Materials and methods

Site and plant material

The experiment was carried out in the research area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, during spring 2023. Eighteen maize

accessions were obtained from the Maize Breeding and Genetics Laboratory of the university: 6159, 6189, 2077, 289, 6160, 345, 2043, 2068, PBG3, 6274, 43, 314, 6202, 2045, 2005, 1066, 113 and 129 (Aslam et al., 2024; Qurban & Muhammad, 2015).

Experimental design and management

The accessions were sown in a randomized complete block design with three replications (Ali et al., 2022). The field was prepared by ploughing and planking. Rows were 75 cm apart, and plants were spaced 25 cm apart within rows. Three rows of non-experimental maize were sown around the trial as a border (Noland et al., 2025). Irrigation, weeding, and fertilization were applied uniformly to all plots, and the crop was harvested at physiological maturity. Cobs were dried and shelled by hand in the laboratory (Ali et al., 2022).

Data recorded

In each plot, three plants were tagged, and their values were averaged. Plant height (cm) was measured from the ground to the tip of the plant at physiological maturity, and ear height (cm) from the ground to the ear-bearing node. Internode diameter (mm) and internode length (cm) were recorded on the same plants. Cob length (cm) was measured on the harvested cobs. Cob diameter (mm) and pith diameter (mm) were measured with a vernier caliper. The number of rows per cob and the number of seeds per row were counted by hand. The weight of 100 grains (g) was taken on an analytical balance (Elangovan et al., n.d.; Qurban & Muhammad, 2015).

Statistical analysis

Data for each trait were analyzed by analysis of variance for a randomized complete block design, following the model $Y_{ij} = \mu + r_i + g_j + e_{ij}$, where r_i is the replication effect, g_j the accession effect, and e_{ij} the error. The analyses were run in

Statistix 8.1, and accession means were compared with Tukey's HSD test at the 5% level.

Variance components were taken from the expected mean squares, with r as the number of replications. Genotypic variance (σ^2_g) was $(MS_g - MSe) / r$, error variance (σ^2_e) was MSe , and phenotypic variance (σ^2_p) was the sum of the two. The genotypic and phenotypic coefficients of variation (GCV and PCV) were the square root of the corresponding variance divided by the grand mean, multiplied by 100. Broad-sense heritability (h^2) was σ^2_g / σ^2_p multiplied by 100. Genetic advance (GA) was $k \times h^2 \times \sqrt{\sigma^2_p}$ with $k = 2.06$ for 5% selection intensity, and was also expressed as a percentage of the mean (GAM). Heritability was classed as high above 60%, moderate from 30 to 60%, and low below 30%. GAM was classed as high above 20%, moderate from 10 to 20%, and low below 10%. GCV and PCV were classed with the same 20% and 10% cut-offs.

Phenotypic correlation coefficients were calculated for all pairs of traits and tested at the 5 and 1% levels. For the multivariate analysis, the accession means were standardized, and a principal component analysis was run on the correlation matrix, keeping components with eigenvalues above a threshold. The same standardized means were clustered with Ward's method on Euclidean distance, and the tree was cut at four clusters. The multivariate calculations were done in Python (NumPy and SciPy).

Results

Variation among accessions

Accessions differed significantly for all ten traits (Table 1). Nine traits had P values below 0.01. Internode diameter was the exception, with a significant but weak F value ($P = 0.049$)

Trait	Unit	Mean	Range of accession means	MS accessions	MS error	F	CV (%)
Plant height	cm	156.0	128.6 to 199.3	1109.67	20.36	54.49**	2.9
Ear height	cm	87.9	69.3 to 135.5	708.47	217.37	3.26**	16.8
Internode diameter	mm	22.6	18.0 to 26.8	14.23	7.33	1.94*	12.0
Internode length	cm	17.7	13.5 to 21.9	16.11	6.31	2.55**	14.2
Cob length	cm	19.8	12.4 to 23.0	21.19	7.20	2.94**	13.6
Rows per cob	no.	17.4	14.1 to 23.7	19.33	4.68	4.13**	12.5
Seeds per row	no.	30.9	27.3 to 36.0	27.56	7.65	3.60**	9.0
Cob diameter	mm	40.1	32.2 to 46.5	66.42	9.09	7.31**	7.5
Pith diameter	mm	12.5	5.9 to 17.3	29.54	9.47	3.12**	24.5
100-grain weight	g	32.5	22.4 to 40.1	52.87	17.65	3.00**	12.9

Table 1. Analysis of variance summary for ten traits in eighteen maize accessions (17 degrees of freedom for accessions, 34 for error)

* Significant at $P < 0.05$; ** significant at $P < 0.01$. MS, mean square; CV, coefficient of variation of the error. Plant height gave the clearest separation. Accession 6160 was the tallest (199.3 cm) and stood alone in

the top Tukey group, while 1066 was the shortest (128.6 cm). Accession 6160 also had the highest ear placement (135.5 cm). Table 2 gives the highest and lowest accessions for each trait.

Trait	Three highest accessions	Lowest accession
Plant height	6160 (199.3), 2077 (182.9), 289 (182.1)	1066 (128.6)
Ear height	6160 (135.5), 6159 (101.1), 2068 (98.6)	2045 (69.3)
Internode diameter	289 (26.8), 345 (25.4), 2077 (25.4)	113 (18.0)
Internode length	345 (21.9), 6160 (21.3), 129 (20.5)	6202 (13.5)
Cob length	6159 (23.0), 6202 (22.7), 6160 (22.5)	129 (12.4)
Rows per cob	2077 (23.7), 6189 (20.3), 113 (20.1)	6159 (14.1)
Seeds per row	6189 (36.0), 289 (36.0), 6159 (35.0)	1066 (27.3)
Cob diameter	2077 (46.5), 289 (44.9), PBG3 (44.8)	129 (32.2)
Pith diameter	2068 (17.3), 113 (16.1), 345 (15.6)	6202 (5.9)
100-grain weight	2068 (40.1), 43 (38.6), 314 (37.1)	6159 (22.4)

Table 2. Accessions with the highest and lowest means for each trait (mean in brackets; units as in Table 1).

For 100-grain weight, accession 2068 was the heaviest (40.1 g), followed by 43 (38.6 g), 314 (37.1 g), and 113 (36.2 g). Accession 6159 was the lightest (22.4 g) and differed

significantly from all four. It shows how component traits can pull apart, since it combined the longest cobs with the fewest rows and the lightest grains.

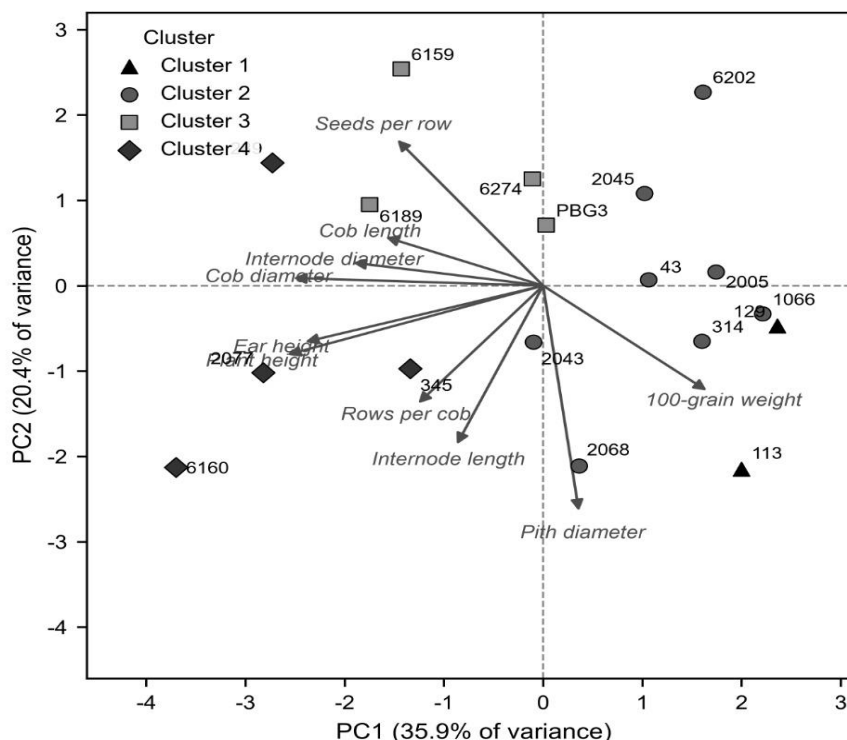


Figure 1. Mean plant height (a) and 100-grain weight (b) of 18 maize accessions evaluated under spring conditions in Faisalabad, Pakistan. Different letters above bars indicate significant differences according to Tukey's HSD test ($P < 0.05$).

Genetic parameters

Table 3 gives the variance-based estimates. Phenotypic variation exceeded genotypic variation for every trait, but the size of the gap differed a great deal. For plant height, the two coefficients were almost the same (GCV 12.2%, PCV 12.6%)

and heritability was 95%. Cob diameter also had high heritability (68%). The remaining traits, except internode diameter, had moderate heritability, between 34% and 51%. Internode diameter had the lowest values for every parameter (heritability 24%, GAM 6.8%).

Trait	GCV (%)	PCV (%)	Heritability (%)	GAM (%)
Plant height	12.2	12.6	95	24.5
Ear height	14.6	22.2	43	19.7
Internode diameter	6.7	13.8	24	6.8
Internode length	10.2	17.5	34	12.3
Cob length	10.9	17.4	39	14.1
Rows per cob	12.7	17.8	51	18.7
Seeds per row	8.3	12.2	46	11.7
Cob diameter	10.9	13.3	68	18.5
Pith diameter	20.6	32.1	41	27.3
100-grain weight	10.6	16.7	40	13.7

Table 3. Genetic parameters for ten traits in eighteen maize accessions.

GCV, genotypic coefficient of variation; PCV, phenotypic coefficient of variation; GAM, genetic advance as a percentage of the mean.

Genetic advance as a percentage of the mean was high for pith diameter (27.3%) and plant height (24.5%), and moderate for ear height, rows per cob, cob diameter, cob length, 100-grain weight, internode length and seeds per row

(11.7 to 19.7%). Plant height was therefore the only trait with both high heritability and high genetic advance. Pith diameter had the highest GCV (20.6%), but also the largest PCV (32.1%) and error CV (24.5%).

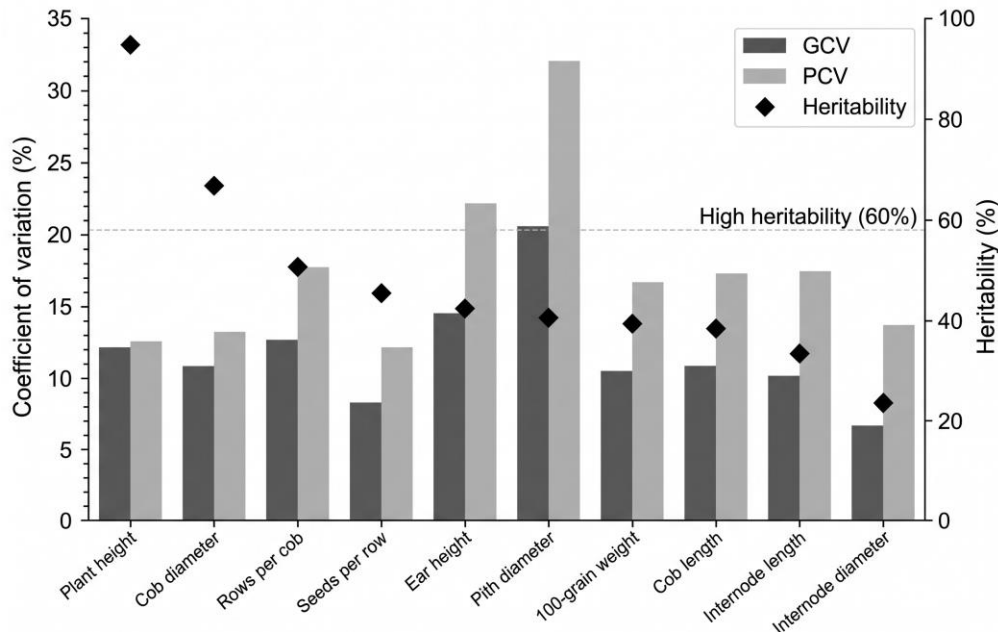


Figure 2. Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), and broad-sense heritability (h^2) for ten traits in 18 maize accessions.

Associations among traits

Most of the significant correlations were among plant-size and ear traits (Table 4). Plant height was positively correlated with ear height ($r = 0.59$), cob diameter ($r = 0.58$), internode

length ($r = 0.44$), rows per cob ($r = 0.42$), and seeds per row ($r = 0.27$). Cob diameter was also correlated with ear height ($r = 0.48$), seeds per row ($r = 0.39$), internode diameter ($r = 0.29$), and cob length ($r = 0.29$). Internode length was

positively correlated with pith diameter ($r = 0.39$), and seeds per row was negatively correlated with pith diameter ($r = -0.30$).

Three traits were significantly correlated with 100-grain weight. Plant height was negatively correlated with it ($r =$

-0.33). Seeds per row ($r = 0.30$) and ear height ($r = 0.29$) were positively correlated with it. Cob length, cob diameter, rows per cob, the internode traits, and pith diameter showed no significant association with grain weight.

	PH	EH	INTD	IL	CL	RCOB	SR	CD	PD
EH	0.589**								
INTD	0.215	0.090							
IL	0.441**	0.231	-0.011						
CL	0.232	0.179	0.288*	-0.103					
RCOB	0.416**	0.266	0.185	0.010	0.027				
SR	0.274*	0.115	0.185	-0.085	-0.066	0.113			
CD	0.577**	0.483**	0.286*	0.060	0.289*	0.256	0.390**		
PD	0.052	0.120	-0.158	0.395**	-0.153	0.126	-0.302*	0.078	
GW	-0.332*	0.286*	-0.154	-0.143	-0.261	0.009	0.302*	0.231	0.187

Table 4. Phenotypic correlation coefficients among ten traits.

PH, plant height; EH, ear height; INTD, internode diameter; IL, internode length; CL, cob length; RCOB, rows per cob; SR, seeds per row; CD, cob diameter; PD, pith diameter; GW, 100-grain weight. * $P < 0.05$; ** $P < 0.01$.

Principal components and clusters

Four principal components had eigenvalues above 1 and together explained 80.3% of the variation (35.9, 20.4, 12.1, and 11.9% for components 1 to 4). The first component had strong negative loadings for plant height (-0.86), cob diameter (-0.84), and ear height (-0.80), and a positive

loading for 100-grain weight (0.55). Accessions with tall plants, high ears, and thick cobs therefore sat at one end, and accessions with heavier grains at the other. The second component was driven by pith diameter (-0.88), internode length (-0.62), and seeds per row (0.57).

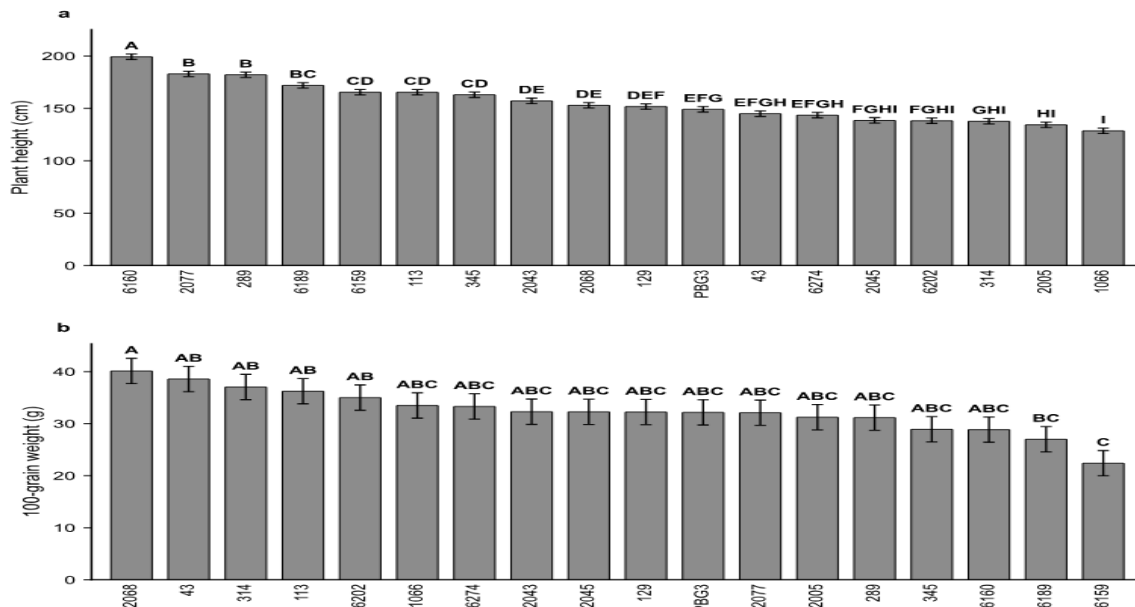


Figure 3. Principal component analysis (PCA) biplot showing the distribution of 18 maize accessions across four cluster groups and trait vectors for PC1 and PC2.

Ward clustering produced four groups (Table 5). Cluster 4 (2077, 289, 6160, 345) held the tallest plants, the highest ears and the thickest cobs, but its grain weight was only 30.3 g. Cluster 2 was the shortest group (141.6 cm) and had the highest mean grain weight (35.0 g); it contained 2068, 43,

Cluster	Accessions	PH	EH	INTD	IL	CL	RCOB	SR	CD	PD	GW
1	113, 129	158.6	75.6	18.6	19.9	15.2	17.6	29.0	33.0	13.7	34.2
2	2043, 2068, 43, 314, 6202, 2045, 2005, 1066	141.6	80.0	22.5	16.9	20.1	16.5	29.2	38.1	13.1	35.0
3	6159, 6189, PBG3, 6274	157.6	94.3	21.7	15.8	19.1	17.5	34.7	43.3	10.6	28.7
4	2077, 289, 6160, 345	181.8	103.4	25.5	20.1	22.1	18.9	31.6	44.5	12.9	30.3

Table 5. Cluster means for ten traits (units as in Table 1). Trait abbreviations as in Table 4.

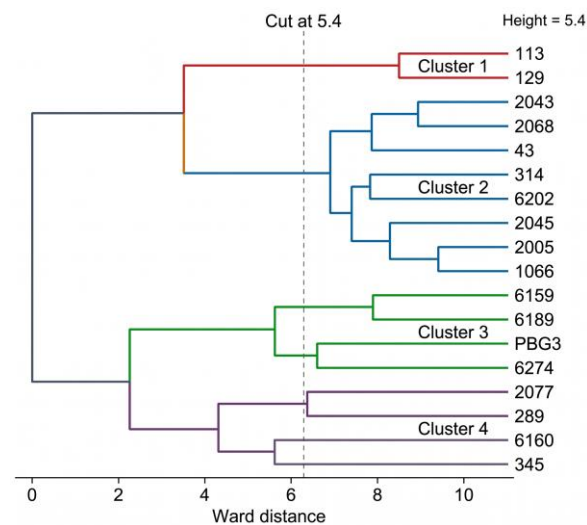


Figure 4. Dendrogram of 18 maize accessions generated from Ward's hierarchical clustering based on standardized morphological and grain traits.

Discussion

The accessions differed for every trait, but the strength of the genetic signal varied. Plant height separated the accessions most clearly ($F = 54.5$, CV 2.9%). Its heritability of 95% and genetic advance of 24.5% of the mean suggest that selection for height in this material should respond well. High heritability together with high genetic advance for plant height is a pattern often seen in maize variability work (Tadesse et al., 2018b). Cob diameter came next, with high heritability and a moderate genetic advance (V. Hemalatha et al., 2020).

The other traits behave differently. Ear height, internode length, cob length, and pith diameter each showed a wide gap between PCV and GCV and heritability of only 34 to 43%, so a large share of the variation seen in the field was not genetic. For ear height, this matches the large error variance (CV 16.8%), which also explains why Tukey's test could separate only a few accessions from the rest. Pith diameter had the highest GCV and genetic advance, but its heritability

and 314. Cluster 3 (6159, 6189, PBG3, 6274) had the most seeds per row (34.7) but the lightest grains (28.7 g). Cluster 1 held only 113 and 129, which share narrow internodes and small cob diameter but differ strongly in cob length (17.9 and 12.4 cm).

of 41% and error CV of 24.5% make selection on it less certain than the genetic advance alone would suggest (Shrestha et al., 2023). Internode diameter gave the weakest result, with a marginal F-test, 24% heritability, and 6.8% genetic advance, and it should not be relied on for selection (Aslam et al., 2024).

These heritability values are screening estimates. They come from one season at one site, so genotype-by-environment interaction cannot be separated from genetic variance and the true heritability may be lower. They also rest on three replications and three plants per plot, which leaves a large error variance for traits measured on single plants (Aslam et al., 2024; Shrestha et al., 2023).

The negative association between plant height and 100-grain weight observed in this study may reflect differences in the genetic backgrounds of the evaluated accessions; however, this relationship requires further investigation in a broader range of germplasm. Taller plants in this set carried higher ears and thicker cobs (cob diameter $r = 0.58$, ear height $r = 0.59$) but not heavier grains. Whether this reflects a real trade-off or only the make-up of these accessions cannot be settled with the present data. Seeds per row were positively correlated with grain weight ($r = 0.30$), so accessions with more kernels per row did not, on the whole, have lighter kernels. A positive link between ear height and grain-weight traits has been reported in other maize material, but the coefficient here is small ($r = 0.29$) and is not strong enough to guide selection. The non-significant correlations of grain weight with cob length and cob diameter mean that these cob traits cannot stand in for grain weight in this material, and accession 6159, with the longest cobs and the lightest grains, shows the same thing (Tadesse et al., 2018b).

The principal components and clusters give a compact picture of the collection (Mohammadi & Prasanna, n.d.). The first component set plant and ear size against grain weight, which matches the correlation result. Cluster 2 combined the highest mean grain weight with the shortest plants and included three

of the four heaviest-grained accessions (2068, 43 and 314), which also had plant heights of 138 to 153 cm. These three are sensible first candidates for use as parents when shorter plants with heavy grains are wanted. Accession 2077 stood out for rows per cob (23.7) and cob diameter, and 6189 for seeds per row (36.0). With only 18 means, the clusters are exploratory, and they say nothing about combining ability or heterosis (Javaid et al., 2023).

Several limits apply. Grain yield per plant was not recorded, so 100-grain weight is the only direct grain trait, and the study cannot rank accessions for yield. The trial covers one season and one location. The next step is a multi-location trial that measures grain yield and flowering time, ideally with molecular markers to check whether the phenotypic groups hold (Erenstein et al., 2022).

Conclusion

Plant height was the only trait with both high heritability (95%) and high genetic advance (24.5% of the mean), so selection for height in this material should respond well. Cob diameter combined high heritability with moderate advance. Grain weight was negatively associated with plant height and positively with seeds per row and ear height. Overall, accessions 2068, 43 and 314 are promising candidates for maize breeding because they combine high 100-grain weight with below-average plant height, while accessions 2077 and 6189 may provide useful traits for improving rows per cob and seeds per row. Evaluating these accessions across multiple environments for grain yield and trait stability will help determine their suitability as parents in future maize improvements programmes.

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